Rapid Discrimination of Citrus reticulata ‘Chachi’ by ESI-IM-HRMS

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A common idea is that some dishonest businessmen often disguise Citrus reticulata Blanco varieties as Citrus reticulata ‘Chachi’, which places consumers at risk of economic losses.

Keywords: Citri reticulatae pericarpium ; Citrus reticulata ‘Chachi’ ; polymethoxylated flavones ; isomers

1. Introduction

Citri reticulatae pericarpium (CRP) is traditional Chinese food medicine, which derives from the dry and ripe peel of *Citrus reticulata* Blanco or its cultivars. The original CRP plants listed in the Pharmacopoeia of the People’s Republic of China mainly include *C. reticulata* ‘Chachi’, *C. reticulata* ‘Dahongpao’, *C. reticulata* ‘Unshiu’, and *C. reticulati* ‘Tangerina’. The peel is harvested, split into three pieces, and dried in the sun [1]. *C. reticulata* ‘Chachi’ produced in Xinhui, China (called “Guangchenpi”, GCP) is considered as a pre-eminent geoherb exhibiting a superb quality and high efficacy [2]. Due to its aroma and utility, GCP is commonly used to make soups, sweetmeats, snacks, and teas, such as ‘Spicy Orange Beef’, ‘Ganpu Tea’, and ‘Tangerine Power’ [3][4]. However, CP (other varieties called “Chenpi”, CP) struggles to maintain the appealing characteristics of GCP. The commercial value of CP is far less than that of GCP. But the frequent phenomenon that CP is a fake of GCP by some greedy businessmen to gain high but illegal profit has been banned repeatedly. Thus, there is an urgency to establish a simple, efficient, and reliable method to distinguish between GCP and CP.

2. Methods in Distinguishing *Citrus Reticulata* ‘Chachi’ from *Citri Reticulatae* Pericarpium

It is hard to distinguish between GCP and CP correctly for consumers, placing them at risk of economic losses. Macroscopical identification is a traditional method of identifying GCP that is based on materials, texture, appearance, size of section characteristics, smell, and color. During identification, the method requires the rich experience of the discriminator rather than advanced and costly instruments; this is a fast, convenient, and widely used method. Hence, macroscopical identification is very popular among people who trade constantly in the market. Nevertheless, this form of identification has several noticeable drawbacks: well-experienced specialists are necessary and the most personal judgments are extremely subjective. Therefore, a unified, clear, and quantitative standard to help average consumers distinguish between GCP and CP is necessary. Recently, it was reported that various techniques were applied to identify GCP, such as electronic nose [5], electronic tongue [6], near-infrared spectroscopy [7], DNA brocade [8], or a combination of those methods. Moreover, fingerprint methodology and the metabolomics approach were also applied to identify GCP [9][10]. Additionally, recent studies reported that the chemical components of GCP mainly included volatiles, flavonoids, alkaloids, and phenolic acids [11][12][13][14]; most studies concentrated on volatiles [15][16] and flavonoids [17][18][19][20]. Rich in volatiles, *C. reticulata* Blanco and *C. reticulata* ‘Chachi’ were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) in several laboratories over the past decades [21][22]. In addition to the volatiles [23], the flavonoids in GCP and CP were also analyzed by liquid chromatography [24][25][26]. Rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry was also employed to identify a total of 41 chemical constituents in CRP [27]. Furthermore, thin-layer chromatography was adopted to identify GCP [28]. As a powerful separation technique, chromatography routinely takes dozens of minutes to complete a cycle [29]. Therefore, it is necessary to establish a rapid method to separate the compounds in GCP.

Ion mobility spectrometry (IMS) [30] is a rapid separation technique on a second timescale [31]. The mechanism involves ions driven by an electric field in a gas damping environment that have a different migration rate. The ions can be separated by their charge state, size, shape, charge position, or structural rigidity [32]. For sensitive detection, IMS is suitable for the trace detection of some volatile organic compounds, such as narcotics [33], explosives [34], chemical warfare [35], and air pollutants [36]. Since the first commercial IMS was manufactured in the 1960s, it has undergone rapid

growth over the past decades and been used widely in many laboratories. Varied commercial IMS instruments were manufactured, such as the drift tube ion mobility spectrometry (DTIMS) [37], traveling wave ion mobility spectrometry (TW-IMS) [38], cyclic ion mobility spectrometry (cIMS) [39], and trapped ion mobility spectrometry (TIMS) [40]. IMS can also be used in combination with chromatography. For instance, headspace–gas chromatography–ion mobility spectrometry was performed to effectively distinguish *C. reticulata* 'Chachi' [41]. Even though the pre-treatment was not required, it still took more time to separate analytes by GC and IMS, respectively. Fortunately, IMS can be flexibly hyphenated with various ionization sources under atmospheric pressure. Electrospray ionization (ESI) is a soft ionization technique that has already been successfully coupled with the IM-MS instrument [42]. Moreover, IM-MS solved the problem that MS was limited for distinguishing isomeric species. The ion's mass-to-charge ratio (*m/z*) and average collision cross-section (CCS) can be obtained, which leads to the rising popularity in many fields, including natural products [43][44], microorganisms [45], carbohydrates [46][47], lipidomics [48][49][50], proteomics [51][52], food [53], and environmental samples [54][55]. With current advances in apparatus, IMS is used as a tool in analytical and bioanalytical applications, rather than as a detector for chemical warfare agents and explosives. The recent development tendency of the ion mobility analyzer is toward a higher performance for completing the increasing measurement task complexity, especially for ultra-high resolutions (>ca. 200) [56]. The U-shaped mobility analyzer (UMA) achieved a resolution of about ca. 180 for single-charge small organic molecules, and up to ca. 370 for multiple-charge +15 myoglobin [57]. Additionally, there is an alternative strategy for identifying isomers of little difference via UMA.

3. Conclusions

The analytical method of distinguishing GCP and CP are introduced. It is well-known that GCP is the dried and mature peel of *Citrus reticulata* 'Chachi'. It is mainly produced in Jiangmen City, Guangdong Province, China, which is a local medicinal material with high price. The price of CP is low, and the phenomenon that some dishonest businessmen often disguise *Citrus reticulata* Blanco or its varieties as *Citrus reticulata* 'Chachi'should be prohibited. Besides, it is necessary to establish a simple and rapid method to distinguish the two kinds of medicinal materials.

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