Comparative study on main components and detection methods of Pericarpium Citri Reticulatae from different habitats

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Abstract. There are many chemical constituents in Pericarpium Citri Reticulatae, such as flavonoids, volatile oil, alkaloids and so on. This paper compares, analyzes and summarizes the main components of Pericarpium Citri Reticulatae from different producing areas and different detection methods. The results showed that although they all have the same chemical composition, there are also differences between them, which leads to the difference of medicinal effect. The contents of hesperidin and heptoxyflavone in Fujian, Sichuan Dahongpao and Wenzhou tangerine were the highest. There are four main types of detection methods of tangerine peel, which are spectrophotometry, electrochemical method, chromatography, gas chromatography-mass spectrometry. When the content of total flavonoids in Pericarpium Citri Reticulatae is measured, spectrophotometry is often used. Both electrochemical method and chromatographic method can be used to determine the content of specific flavonoids. Chromatography can also be used for the separation of flavonoids and the identification between Pericarpium Citri Reticulatae and Citrus reticulate Blanco‘chachi’ (Guangchenpi). However, gas chromatography-mass spectrometry (GC-MS) is mostly used for the determination of volatile compounds in Pericarpium Citri Reticulatae. With the development of science and technology in the future, it will be more in-depth to explore the composition differences of Pericarpium Citri Reticulatae from different habitats, and more efficient detection methods will be used for research.

1. Introduction

Pericarpium Citri Reticulatae is the dried and mature pericarp of Citrus reticulate Blanco and its cultivated varieties. It is a kind of medicine and food homologous medicine. It is produced in Fujian, Zhejiang, Guangdong, Guangxi, Jiangxi, Hunan, Guizhou, Yunnan, Sichuan and other places. It is used to treat symptoms such as abdominal distension and fullness, lack of food and vomiting and diarrhea, cough and phlegm, etc. It has the effect of regulating qi and invigorating spleen, dryness and dampness and reducing phlegm [1].

In this paper, based on the domestic literatures, the content of chemical components of Pericarpium Citri Reticulatae from Xinhui, Sichuan, Wenzhou and Fujian were compared to provide scientific data for the quality evaluation of Pericarpium Citri Reticulatae.
2. Main chemical constituents of Pericarpium Citri Reticulatae

Modern pharmacological studies show that the chemical constituents of Pericarpium Citri Reticulatae is complex, and about 140 chemical substances have been isolated and identified at present [2]. The main chemical substances are flavonoids, essential oils, alkaloids, polysaccharides, etc. while the types and contents of chemical components in Pericarpium Citri Reticulatae from different areas are different [3].

2.1. Flavonoids

Song et al. [4] used HPLC to determine the content of flavonoids in different sources of Pericarpium Citri Reticulatae. Wei et al. used high performance liquid chromatography (HPLC) to determine the content of hesperidin, nobiletin, tangerine and synephrine, the main active components of Pericarpium Citri Reticulatae [5].

2.2. Volatile oils

Sheng et al. used gas chromatography-mass spectrometry (GC-MS) to determine the main chemical components and contents of the volatile oil from Pericarpium Citri Reticulatae in different areas [6]. Among the identified compounds, Pericarpium Citri Reticulatae from different regions contains limonene, α-pinene, terpenes, etc. Luo et al. used conventional physical and chemical methods to study the raw materials of Xinhui Pericarpium Citri Reticulatae and three typical raw materials of Pericarpium Citri Reticulatae [7]. Some active components were detected in advance, which provided scientific basis for the development of Pericarpium Citri Reticulatae resources. Pei-kun through the establishment of the content determination method of the effective components of Pericarpium Citri Reticulatae, combined with pharmacological experiments, the volatile oil of four kinds of Pericarpium Citri Reticulatae was determined by GC-MS and compared with pharmacological experiments [8].

2.3. Alkaloids

Through thin-layer chromatographic scanning (TLCS), Huang Ai-dong found that the peel of eight varieties contained symphorin and N-methyl-tyramine, respectively 0.02 - 0.10% and 0.010 ~ 0.030% [10]. However, the variety with the lowest content of symphorin and N-methyltyramine was Xinhui Pericarpium Citri Reticulatae.

2.4. Trace elements

Pericarpium Citri Reticulatae is rich in trace elements, mainly selenium and copper elements, with enhanced immunity, anti-aging, maintain hematopoietic function, anti-influenza and other functions. Lin et al. determined various trace elements in Pericarpium Citri Reticulatae by atomic absorption spectrophotometry [11].

2.5. Other Components

In addition to the above flavonoids, volatile oils and alkaloids, pericarp also contains pectin, limonin, phenolic acid and so on. The pectin content in Pericarpium Citri Reticulatae reached 15% - 30%. Li et al. used acid method to extract crude pectin from Pericarpium Citri Reticulatae [12]. Pericarpium Citri Reticulatae is also rich in phenolic acid [13]. Yang et al. reported that the total content of phenolic acid in Xinhui Pericarpium Citri Reticulatae was 2.112%, among which the content of gallic acid and limonin were 0.0006% and 0.0998% [14]. Respectively, with antioxidant, antibacterial, anticancer and other physiological functions. Table 1 shows the determination results of main compounds in Pericarpium Citri Reticulatae from different areas [15, 16].

Table 1
inconsistency of their main chemical components, their pharmacological effects are also different.

With Xinhui Pericarpium Citri Reticulatae was found to have a great difference in the content of synephrine, followed by Fujian tangerine, Sichuan tangerine and Xinhui Pericarpium Citri Reticulatae. However, Xinhui Pericarpium Citri Reticulatae was found to have a great difference in the content of synephrine, which was 0.20%. The content of synephrine in the other three areas was similar.

Therefore, the pharmacological effects of the four drugs are generally the same, but due to the inconsistency of their main chemical components, their pharmacological effects are also different.

**Table 1.** Determination of main compounds in Pericarpium Citri Reticulatae from different areas

| Component | Place of Origin | Xinhui Pericarpium Citri Reticulatae | Sichuan Tangerine | Wenzhou Citrus unshiu | Fujian Tangerine | References |
|-----------|----------------|--------------------------------------|------------------|-----------------------|-----------------|-----------|
| Hesperidin |                | 3.54-8.82%                          | 3.59-8.30%       | 4.40-11.29%            | 5.62-11.29%     | [5,7]     |
| Nobiletin  |                | 0.13-0.56%                          | 0.15-3.31%       | 0.03-1.61%             | 0.04-1.32%      | [5,7]     |
| 7-Methoxy-2-phenyl-4H-chromen-4-one |          | 0.01-0.04%                          | 0.01-0.04%       | 0.01-0.40%             | 0.01-0.6%       | [5,7]     |
| Tangeretin |                | 0.07-0.44%                          | 0.09-0.06%       | 0.01-0.84%             | 0.02-0.76%      | [5,7]     |
| D-limonene |                | 75.77-79.70%                        | 82.50-86.07%     | 84.31-78.26%           | 83.55%          | [6,7,8]   |
| γ-Terpinene|                | 11.06-15.74%                        | 4.70-7.61%       | 5.12-7.99%             | 4.86-4.98%      | [6,7,8]   |
| α-Pinene   |                | 2.23-2.48%                          | 1.07-1.33%       | 0.88-1.26%             | 1.08-1.03%      | [6,7,8]   |
| β-Pinene   |                | 1.32-1.75%                          | 0.41-0.17%       | 0.10-0.86%             | 0.48-0.45%      | [6,7,8]   |
| β-Mycene   |                | 1.10-2.13%                          | 1.61-2.46%       | 0.65-2.11%             | 1.60-2.08%      | [6,7,8]   |
| Terpinene oil |            | 1.18%                               | 0.48%            | 0.46%                  | 0.29%           | [6,7]     |
| β-Elemene  |                | 0.05%                               | 0.21%            | 0.02%                  | 0.04%           | [8]       |
| Germacrene |                | 0.01%                               | 0.24%            | 0.11%                  | 0.01%           | [8]       |
| Citronellal|                | 0-0.01%                             | 0.08-0.11%       | 0.00%                  | 0-0.04%         | [6,7,8]   |
| g-Elemene  |                | 0.02%                               | 0.24%            | 0.02%                  | 0.04%           | [8]       |
| Synephrine |                | 0.20%                               | 0.30%            | 0.49%                  | 0.35%           | [5,10,17] |
| Linalool   |                | 0.13%                               | 2.42%            | 0.43%                  | 0.34%           | [6]       |

Although the dried Pericarpium Citri Reticulatae from Xinhui, Sichuan, Wenzhou and Fujian provinces has the same chemical composition, there are also differences.

Among the flavonoids, the content of hesperidin and 7-Methoxy-2-phenyl-4H-chromen-4-one in Fujian tangerine was the highest, 5.62%-11.29% and 0.01%-0.6% respectively. Sichuan tangerine had the highest content of tangerine peel, ranging from 0.15% to 3.31%. The content of tangerine peel was the highest in Wenzhou Citrus unshiu, ranging from 0.01% to 0.84%. In addition, the content of hesperidin in Fujian tangerine is similar to that in Wenzhou Citrus unshiu, and the content of hesperidin in Xinhui Pericarpium Citri Reticulatae is similar to that in Sichuan tangerine, but the content of hesperidin in the former two is 1.5 times higher than that in the latter two.

Among the four producing areas, the d-limonene content of Wenzhou Citrus unshiu was generally higher than that of the other three, ranging from 0.36% to 10.66%. The content of γ-Terpinene and α-Pinene in Xinhui Pericarpium Citri Reticulatae is about 2-3 times higher than that in other regions. In addition, the Terpinene oil content of Xinhui Pericarpium Citri Reticulatae is 4 times that of Fujian tangerine and 2.5 times of Sichuan tangerine and Wenzhou Citrus unshiu.

The contents of linalol, g-Elemene, germacrene and citronellal in the volatile oil of Sichuan Tangerine were the highest, which were 2.42%, 0.24%, 0.24% and 0.08-0.11%, respectively. The contents of the other three components were extremely low or even none.

The content of synephrine in Pericarpium Citri Reticulatae was the highest in Wenzhou Citrus unshiu, followed by Fujian tangerine, Sichuan tangerine and Xinhui Pericarpium Citri Reticulatae. However, Xinhui Pericarpium Citri Reticulatae was found to have a great difference in the content of synephrine, which was 0.20%. The content of synephrine in the other three areas was similar.

Therefore, the pharmacological effects of the four drugs are generally the same, but due to the inconsistency of their main chemical components, their pharmacological effects are also different.
3. Determination of chemical components in Pericarpium Citri Reticulatae
There are many chemical components that have value as therapeutics in pericarpium citri reticulatae. For the detection of active ingredients, there are four main types of methods: spectrophotometry, electrochemistry, chromatography and gas chromatography-mass spectrometry [18].

3.1. The common detection methods of Pericarpium Citri Reticulatae:
Table 2 shows the different detection methods of Pericarpium Citri Reticulatae.

| Table 2. Different detection methods of Pericarpium Citri Reticulatae |
|--------------------------|------------------|---------------------|------------------|
| Method                   | Type             | Main detection substances and results     | References       |
| Ultraviolet spectrophotometry | Spectrophotometry | Total flavonoids 7.8%~15.1% Selenium 93.8% | [19,20,21]       |
| Fluorescence spectrophotometry | Spectrophotometry | Total flavonoids 7.6% Aflatoxin was not detected or less than 5 μg/kg | [22,23]         |
| Polarography             | Electrochemistry  | Hesperidin 10.8%~12.0%                        | [24]             |
| Capillary electrophoresis | Electrochemistry  | Flavonoids 3.4%~8.7% Hesperidin 3.52%~4.07%   | [25]             |
| Thin layer chromatographic scanning (TLCS) | Chromatography | Nobiletin 0.14%~0.19% Tangeretin 0.10%~0.12% Hesperidin 4.503%~11.25% | [26] |
| High performance liquid chromatography (HPLC) | Chromatography | Polymethoxylated flavones 0.40%~1.51% the difference of different Pericarpium Citri Reticulatae Identification of Pericarpium Citri Reticulatae and Citrus reticulate Blanco’chachi’ (Guangchenpi) | [4,27,28] |
| Fingerprint              | Chromatography   | Identification of volatile compounds         | [29,30,31]       |
| Gas chromatography-mass spectrometry (GC-MS) | Gas chromatography-mass spectrometry (GC-MS) | Identification of volatile compounds | [32,33,34] |

According to the above table, the detection objects of various detection methods are different. When the content of total flavonoids in Pericarpium Citri Reticulatae is measured, spectrophotometry is often used, but spectrophotometry is not suitable for the determination of specific types of flavonoids. Both electrochemical method and chromatographic method can be used to determine the content of specific flavonoids. Chromatography can also be used for the separation of flavonoids and the identification between Pericarpium Citri Reticulatae and Citrus reticulate Blanco’chachi’ (Guangchenpi). However, gas chromatography-mass spectrometry (GC-MS) is mostly used for the determination of volatile compounds in tangerine peel. Among these main detection methods, chromatography is widely used and involves more aspects.

3.2. Ultraviolet spectrophotometry
Zheng et al. [19] determined the content of total flavonoids and polysaccharides in pericarpium citri reticulatae from different areas. The results showed that there was a good linear relationship between total flavonoids and polysaccharides in a certain range, with good reproducibility and accurate results.
Wu et al. [20] used five methods to scan the spectrum in the wavelength range of 200-600 nm, and selected the better spectrophotometry of total flavonoids in pericarpium citri reticulatae. Liu et al. [21] detected the trace element selenium in pericarpium citri reticulatae from different producing areas, and found that there were obvious differences in the content of selenium in different areas of pericarpium citri reticulatae.

3.3. Fluorescence spectrophotometry
Zhang et al. [22] used fluorescence spectrophotometry to determine the total flavonoids of many traditional Chinese medicine, such as pericarpium citri reticulatae. The conclusion is that fluorescence spectrophotometry can be used to determine the total flavonoids of most traditional Chinese medicine except hawthorn.

Li et al. [23] detected the content of aflatoxin in various kinds of pericarpium citri reticulatae from different places in the market by fluorescence spectrophotometry, and found that the content of aflatoxin in all kinds of pericarpium citri reticulatae detected did not exceed the national maximum limit of 5 μg/kg.

3.4. Polarography
Hu et al. [24] analyzed the hesperidin in pericarpium citri reticulatae and found that hesperidin appeared obvious derivative wave peak on polarography.

3.5. Capillary electrophoresis
Tamai (Japan) [25] used capillary electrophoresis to detect flavonoids in Pericarpium Citri Reticulatae, and determined the optimal conditions for the determination of total flavonoids. The results can also be applied to the detection of health products containing flavonoids in pericarpium citri reticulataes.

3.6. Thin layer chromatographic scanning (TLCS)
Zhou et al. [26] used TLCS to determine the content of flavonoids in six kinds of pericarpium citri reticulataes from different areas. The results showed that there was a good linear relationship between hesperidin, nobiletin and tangeretin.

3.7. High performance liquid chromatography (HPLC)
Song et al. [4] analyzed the pericarpium citri reticulataes from different areas, and found that the content of hesperidin in them ranged from 4.503% to 11.25%, which met the requirement of the 2015 edition of Chinese Pharmacopoeia that the content of hesperidin in pericarpium citri reticulatae was more than 3.5%. However, the contents of nobiletin and tangeretin from different habitats were different.

Lv et al. [27] used chromatographic separation method to separate nobiletin and tangeretin with high purity. The establishment of this method laid a material foundation for the screening of bioactivity of pericarpium citri reticulatae flavonoids and in-depth study of in vivo experiments.

Li et al. [28] used chromatography to detect the content of methoxyflavone in pericarpium citri reticulataes from different producing areas. The results showed that the quality of Nanfeng tangerine, Sichuan Red orange and Chongqing Dahongpao were comparable to those of Xinhui tea branch citrus, which could be used for medicinal development. However, due to the low yield of pericarpium citri reticulatae in Jiangxi Province, the research on it is not enough.

3.8. Fingerprint
Xu et al. [29] compared the fingerprints of citrus reticulata blanco’chachi’ from different habitats with that of pericarpium citri reticulatae. The experimental results showed that the fingerprints of citrus reticulata blanco’chachi’and other five varieties of pericarpium citri reticulataes were different.

Yi et al. [30] used fingerprint method to analyze different citrus peels, and compared the fingerprints of pericarpium citri reticulataes from different producing areas and different brands. It was found that
there were obvious differences in fingerprint of different tangerine peel, and some samples also produced new hesperidin peak.

Luo et al. [31] established the fingerprint of citrus reticulata blanco ‘chachi’, compared the similarity of pericarpium citri reticulataes from different places of origin and different dates, and finally established the HPLC fingerprint with common peak, which can further realize the identification of citrus reticulata blanco ‘chachi’ and pericarpium citri reticulatae, with the identification rate of 100%.

3.9. Gas chromatography-mass spectrometry (GC-MS)

Gao et al. [32] detected the volatile compounds of pericarpium citri reticulatae by experiments, and identified 89 kinds of volatile substances, including 44 alkenes, 10 alcohols, 10 aldehydes, 8 phenols, 7 esters, 4 ketones and 6 other substances.

Yan et al. [33] studied the relationship between the change of volatile oil content in pericarpium citri reticulatae and storage time by GC-MS. It was found that the longer the storage time, the lower the molecular weight components and the higher the molecular weight components.

Guo et al. [34] identified and analyzed the volatile components in the essential oil of pericarpium citri reticulatae, and the experiment showed that there were 124 kinds of volatile compounds in the essential oil of tangerine peel.

4. Discussion

The main chemical components of Pericarpium Citri Reticulatae are flavonoids, volatile oil, alkaloids and other components. In this paper, the content of main active components in Pericarpium Citri Reticulatae from different areas were compared. It was found that the content of hesperidin in Fujian tangerine was much higher than that in other areas, with the content of 5.62-11.29%; the content of d-limonene in the volatile oil of Wenzhou Citrus unshiu is 0.36% - 10.66% higher than that in other regions; the Terpinene oil content of Xinhui Pericarpium Citri Reticulatae is 1.18%, which is 4 times of Fujian tangerine and 2.5 times of Sichuan tangerine and Wenzhou Citrus unshiu; The contents of linalool, g-elemene, geranione and citronellal in the volatile oil of Sichuan tangerine were higher than those in the other three components, which were 2.42%, 0.24%, 0.24% and 0.08-0.11%, respectively. The results showed that the contents of some components in Pericarpium Citri Reticulatae from different areas were higher than those from other areas. Therefore, the quality of tangerine peel from different producing areas should be compared and analyzed from the specific efficacy.

After years of research, spectrophotometry, high performance liquid chromatography, gas chromatography-mass spectrometry and other methods have been widely used in the detection of components of pericarpium citri reticulatae, and there are different detection methods for different components of pericarpium citri reticulatae. For example, Spectrophotometry, chromatography and electrochemistry can be used to detect flavonoids; the determination of trace elements in tangerine peel, UV spectrophotometry can be used; the volatile oil in Pericarpium Citri Reticulatae can be used by GC-MS.

In the future, with the continuous development of science and technology, the detection instruments and methods will be more accurate and efficient, and the chemical components in Pericarpium Citri Reticulatae will be further studied and applied. In order to study the effect of different producing areas on the chemical composition of Pericarpium Citri Reticulatae, it should be combined with different storage years and harvest time.

5. Conclusion

To sum up, by comparing different detection methods, the main components of Pericarpium Citri Reticulatae from different habitats were determined, and it was found that there were significant differences in the content of main components in different habitats. Further study on the differences of some components of Pericarpium Citri Reticulatae from different habitats is more conducive to distinguish the differences between different habitats.
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