Determination of Gallic acid, Epicatechin and Epicatechin gallate in Different Varieties, Manufacturers and Grades of Tea by HPLC-DAD

Juan Kong\textsuperscript{1}, Lingling Zhang\textsuperscript{1}, Hua Feng\textsuperscript{2}, Xiaosong Yang\textsuperscript{1}, Xulong Huang\textsuperscript{1}, Xiangpei Wang\textsuperscript{3}, Hongmei Wu\textsuperscript{1}, *

\textsuperscript{1}Department of Pharmacognosy, Guizhou University of Traditional Chinese Medicine, Guiyang City, Guizhou Province, Guizhou 550002, China
\textsuperscript{2}Product quality inspection and Testing Institute of Zunyi, Zunyi City, Guizhou Province, Zunyi 563002, China
\textsuperscript{3}Chengdu University, China

*Corresponding author’s e-mail: whm0425@126.com

Abstract. Tea has been recognized by scientists for its health benefits and disease prevention. But there are many varieties of tea, and its quality is affected by many factors. The quality of tea determines its effects of health care and disease prevention, and gallic acid, epicatechin and epicatechin gallate are the main active ingredients in tea. The goal of this study was to detect the contents of gallic acid, epicatechin and epicatechin gallate in 33 batches of tea from the market by HPLC to explore the effects of these factors on the chemical composition of tea such as different varieties, different manufacturers and different grades. After the analysis of tea samples by HPLC, the relative standard deviations for precision, repeatability, stability and average recovery were acceptable, and the average recovery rates of gallic acid, epicatechin and epicatechin gallate were 100.2, 101.57 and 102.69, respectively. The results showed that gallic acid, epicatechin and epicatechin gallate were detected in both green tea and oolong tea, while epicatechin was not contained in black. The content of gallic acid and epicatechin gallate in green tea was higher than that in oolong and black tea. The composition content of some special grade tea was lower than that of first or second grade tea. Therefore, the results of this study provide a reference for people to choose high-quality tea and reasonable quality control of tea.

1. Introduction

The tea that people drink every day is the processed product of the leaves of tea plant (\textit{Camellia sinensis} (L.) O. kuntze), it is among the most popular beverages consumed worldwide, because it can protect the body, prevent diseases and taste good [1, 2]. Tea contains more than 300 kinds of chemical ingredients beneficial to the human body, mainly containing tea polyphenols [3], tea polysaccharides [4], theanine [5], alkaloids [6] and other compounds, in which tea polyphenols are the main medicinal component in tea, accounting for about 18-36\% of the dry weight of tea. Catechins are the main chemical components in tea polyphenols, the catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin 3 gallate (EGCG) [7]. Modern research shows...
that tea has anti-cancer, anti-oxidation [8], anti-HIV [9], anti-inflammatory, analgesic and other effects [10].

At present, there are many kinds of teas sold in the market. According to the different processing of tea, tea can be divided into green tea, black tea, oolong tea, white tea, yellow tea and reprocessed tea. In the production process, green tea is non-fermented, oolong tea belong to semi-fermented, and black tea is a whole fermented product. Tea have different origins, manufacturers and grades, of which the grade is divided into three to four. The economic value of different grades of tea is different, most people think that the lower the grade of tea is better, and the economic value is also higher [11-13]. Recently, there are many studies on the chemical content of tea. For example, HPLC-MS-MS method was used to simultaneously determine 8 catechins and 4 kinds of theaflavins in green, black and oolong tea. However, it is unclear how the different degrees of processing, manufacturers and grades affect the quality of tea.

In order to further explore the effects of different degrees of processing, manufacturers and grades on the chemical content of tea. This study selected different varieties, manufacturers and grades of green, black and oolong tea, and the content of all samples was determined by HPLC-DAD.

2. Materials and methods

2.1. Sample and reagents

In this experiment, green tea includes Meitan Cuiya, Fenggang zinc selenium, DouYun maojian, Biluochun, West lake longjing, Tianzhu tea and Emerald. Oolong tea has Tie Guanyin and Dahongpao, and a black tea is Zunyi black tea. The origins and grades of all teas are shown in table 6. HPLC-grade solvents (methanol and acetonitrile) were purchased from Tianjin kemio chemical reagent co., LTD (Tianjin, China). Phosphoric acid (Analytical grade) was bought from Chongqing chuandong co. LTD (Chongqing, China), and reference substance (GA and EC) were purchased from China institute of pharmaceutical and biological products identification (China), and ECG was obtained from Chengdu pufeide biotechnology co., LTD (Sichuan, China), their purity is more than 98%.

2.2. Preparation the Sample Solution

33 batches of tea are crushed and filtered in a 60-mesh sieve. The tea sample was precisely weighed to 1.0 g and put into 50 mL round bottom flask, with 75% methanol, and reflux 0.5 h in 80℃ water, repeating it operation once. Finally, merged the twice filtrate obtained and set the volume to 100 ml, shaking it well. All samples were through a 0.22µm filter membrane.

2.3. Preparation of Reference Solution

The standard compounds were dissolved in 75% methanol, making a of 0.2 mg/mL of gallic acid, 0.2 mg/mL of epicatechin, 0.8 mg/mL of epicatechin gallate.

2.4. Chromatographic Procedures

An Agilent 1260 high performance liquid chromatograph (HPLC) system comprising autosampler and DAD detector was used to test all samples. The Agilent Eclipse XDB-C18 column (4.6 mm×250 mm, 5 μm) was adopted for the analysis. The mobile phase consisted of A (0.2% phosphoric acid solution) and B (acetonitrile). The gradient mode was as follows: 5–10% B for 0–15 min; 10–12% B for 15–32 min; 12–18% B for 32–37min; 18–20% B for 37–44 min; 20–22% B for 44–49 min; 22–30% B for 49–57 min. The flow rate was set at 1.0 mL/min. The detection wavelength was 280 nm. The column temperature was set at 30℃ and sample volume was 10 μL.
3. Results

3.1. Linearity analysis
All the reference substances was serially diluted with 75% methanol, to obtain six different concentrations for the three reference curves. The linearity of each analyte was evaluated by plotting calibration curves for different concentrations and corresponding peak areas. The results were shown in Table 1.

Table 1. Linear equations, linear ranges, correlation coefficients of three components

| Analytes | Linear Ranges(mg/mL) | Regression Equations | correlation coefficients ($R^2$) |
|----------|----------------------|----------------------|-------------------------------|
| GA       | 0.0034–0.2125        | $Y=22708X+7.0569$    | 0.9999                       |
| EC       | 0.0065–0.2068        | $Y=7000.3X+15.497$   | 0.9986                       |
| ECG      | 0.0051–0.5130        | $Y=13575X+16.51$     | 0.9998                       |

3.2. The precision test
The reference solution was analyzed for six times, to evaluate the precision. Calculating the RSDs of each chromatographic peak, the results were shown in Table 2, and the RSDs were less than 3%, indicating that the precision of the method was good.

Table 2. Results of precise test (n = 6)

| NO. | Peak area of GA | Peak area of EC | Peak area of ECG |
|-----|-----------------|-----------------|------------------|
| 1   | 2097.23560      | 148.57993       | 3102.28442       |
| 2   | 2103.39209      | 148.29977       | 3131.62646       |
| 3   | 2120.14868      | 149.12741       | 3132.88135       |
| 4   | 2107.53369      | 149.27194       | 3127.86377       |
| 5   | 2116.71655      | 148.80724       | 3139.63086       |
| 6   | 2126.85669      | 146.92459       | 3128.13965       |
| Mean| 2111.98055      | 148.50181       | 3127.07108       |
| RSD(%)| 0.53            | 0.57            | 0.41             |

3.3. The stability test
The S9 solution was tested and analyzed at 1, 4, 8, 10, 12, and 16 h, to calculate the RSDs. The results were shown in Table 3. The RSDs were less than 3%, suggesting that the method was stable within 16 h.

Table 3. Results of stability test (n = 6)

| Time/h | Peak area of GA | Peak area of EC | Peak area of ECG |
|--------|-----------------|-----------------|------------------|
| 1      | 2136.27368      | 133.92334       | 2705.02612       |
| 4      | 2230.5105       | 134.14278       | 2726.45386       |
| 8      | 2243.27856      | 133.38309       | 2750.29956       |
| 10     | 2263.32983      | 133.54245       | 2786.31519       |
| 12     | 2280.00903      | 133.78487       | 2811.29150       |
| 16     | 2297.55005      | 137.07199       | 2821.27075       |
| Mean   | 2241.82527      | 134.30809       | 2766.77616       |
| RSD (%)| 2.55            | 1.03            | 1.70             |
3.4. In the repeatability test
Six duplicates of S9 were extracted and analyzed, according to the sample preparation procedure and the HPLC method. The RSDs of Composition content were calculated, its values were < 4%. The results showed in Table 4.

Table 4. Results of repeatability test (n = 6)

| NO. | Composition content of GA (mg/g) | Composition content of EC (mg/g) | Composition content of ECG (mg/g) |
|-----|---------------------------------|---------------------------------|----------------------------------|
| 1   | 8.74                            | 2.14                            | 21.39                            |
| 2   | 8.59                            | 2.37                            | 21.30                            |
| 3   | 9.13                            | 2.34                            | 22.73                            |
| 4   | 9.03                            | 2.35                            | 21.92                            |
| 5   | 8.71                            | 2.39                            | 21.40                            |
| 6   | 8.58                            | 2.35                            | 21.31                            |
| Mean| 8.80                            | 2.32                            | 21.67                            |
| RSD(%) | 2.60                     | 3.90                            | 2.61                             |

3.5. In the accuracy test
Certain amounts of the three analytes’ standards were added to the tea samples (S9), with the six replicates. Then these three samples were treated, as in the method described above. Recovery rate was used as the evaluation index and calculated as recovery rate (%) = (Found amount - Known amount) × 100%/Added amount. The RSD of the accuracy values of the three components were shown in Table 5, respectively.

Table 5. Recovery test results of gallic acid, epicatechin and epicatechin gallate in tea (n=6)

| Analyte        | Quality(g) | Sample content (mg/g) | Standard quality (mg) | Measured content (mg/g) | Recovery (%) | Mean recovery (%) | RSD (%) |
|----------------|------------|-----------------------|-----------------------|-------------------------|--------------|-------------------|--------|
| gallic acid    | 0.250      | 4.4145                | 3.5316                | 8.0521                  | 103          |                   |        |
|                | 0.250      | 4.4109                | 3.5288                | 7.9759                  | 101.03       |                   |        |
|                | 0.250      | 4.4092                | 4.4092                | 8.8209                  | 100.06       |                   |        |
|                | 0.250      | 4.418                 | 4.418                 | 9.011                   | 103.96       |                   |        |
|                | 0.250      | 4.4074                | 5.2889                | 9.4895                  | 96.09        |                   |        |
|                | 0.250      | 4.4127                | 5.2952                | 9.5522                  | 97.06        |                   |        |
| epicatechin    | 0.250      | 1.083                 | 0.2002                | 1.9679                  | 102.14       |                   |        |
|                | 0.250      | 1.0834                | 0.2002                | 1.9741                  | 102.76       |                   |        |
|                | 0.250      | 1.0826                | 0.2501                | 2.1965                  | 102.9        |                   |        |
|                | 0.250      | 1.0839                | 0.2504                | 2.1754                  | 100.71       |                   |        |
|                | 0.250      | 1.0834                | 0.3004                | 2.3589                  | 98.11        |                   |        |
3.6. Analysis of tea samples

The content of GA, EC and ECG of 33 batches of tea samples were determined by HPLC. Among the teas produced by different types, grades and manufacturers, the content of GA, EC and ECG were different. The results were shown in Table 6. In different varieties of tea, GA, EC and ECG were detected in both green tea and oolong tea, but EC was not detected in black tea. In addition, the highest GA and ECG content was green tea. In different green tea, the content of GA and ECG in Meitan Cuiya tea and Douyun maojian tea was higher than that in other green tea, but the content of EC in Meitan Cuiya tea was lower. In oolong tea, the content of GA in Dahongpao tea was higher than that in Tie Guanyin tea, but the content of EC and ECG was lower. In different grade tea, the content of GA, EC and ECG in the first-grade of Meitan Cuiya tea was higher than that of the special grade. In the second grade of Douyun maojian tea, the content of ECG was the highest. There was no significant difference in the contents of GA, EC and ECG in Fenggang zinc selenium tea, Tie Guanyin, Emerald and Zunyi black tea. In different sources of tea, the higher content of GA, EC and ECG was Yongxing tea planting base, in Meitan Cuiya tea. And the difference of three components in Fenggang zinc selenium tea and Tie Guanyin was not obvious.

Table 6. Contents of GA, EC and ECG in 33 batches of tea of different species and grades

| NO. | Sample name       | Grade        | Source                          | GA content (mg/g) | EC content (mg/g) | ECG content (mg/g) |
|-----|-------------------|--------------|---------------------------------|-------------------|-------------------|--------------------|
| S1  | Meitan Cuiya tea  | Special grade| Yongxing tea planting base      | 6.51              | 3.17              | 22.07              |
| S2  | Meitan Cuiya tea  | First grade  | Yongxing tea planting base      | 9.17              | 3.32              | 29.67              |
| S3  | Meitan Cuiya tea  | Second grade | Yongxing tea planting base      | 8.59              | 3.07              | 25.40              |
| S4  | Meitan Cuiya tea  | Special grade| Li Xiang's Tea Factory          | 7.49              | 2.16              | 19.08              |
| S5  | Meitan Cuiya tea  | First grade  | Li Xiang's Tea Factory          | 6.81              | 3.61              | 23.59              |
| S6  | Meitan Cuiya tea  | Second grade | Li Xiang's Tea Factory          | 7.60              | 3.58              | 24.65              |
| S7  | Meitan Cuiya tea  | Special grade| Chunjiang Tea Co., Ltd          | 5.98              | 2.71              | 19.76              |
| S8  | Meitan Cuiya tea  | First grade  | Chunjiang Tea Co., Ltd          | 6.52              | 3.00              | 21.77              |
| S9  | Meitan Cuiya tea  | Second grade | Chunjiang Tea Co., Ltd          | 9.07              | 2.37              | 22.77              |
4. Discussion
Modern research has shown that EC has the function of lowering blood pressure, changing metabolic and hemorheological properties and crossing the blood-brain barrier, which is beneficial to cardiovascular and neurological health. The results of this experiment show that if you want to prevent cancer and cardiovascular diseases by eating tea or tea extract, you can't eat black tea. The best choice is green tea. In the tea market, the economic value of the special-grade tea is higher than that of the first and second grades, but the study shows that the content of GA, EC and ECG in the special-grade tea is lower than the first or second grade, and the above three chemical components are one of the active ingredients of tea. Therefore, whether the quality of special grade tea is positively related to its economic value remains to be discussed.
5. Conclusion
The analytical method of the experiment was simple, stable and reproducible, and could be used for quality control of different teas. So using this method to study different varieties, manufacturers and grades of tea, in order to explore the quality of different teas. The results showed that black tea had no EC, while green tea had the highest contents of GA and ECG. In different grades of tea, the content of GA, EC and ECG in special grade tea was lower than that of first grade and second grade tea. As a widely consumed beverage, tea is closely related to people’s health. Therefore, it is necessary to take measures to strengthen the quality control of tea.

Acknowledgements
This work was supported by Science and Technology Project of Guizhou Province [Qian Ke he (2019) NO.2385] and Innovative Talent Team Training Project for Specialty Foods and Chinese Medicinal Materials in Zunyi City, Guizhou Province, China [ Zun Shi Ke he (2016) NO.7].

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