Comparison of Catechins and Antioxidant Activity in Four kinds of Sichuan tea

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Abstract: Catechins of the nine representative teas produced in Sichuan, which belonged to green tea, yellow tea, dark tea and black tea, were determined by UHPLC. Their antioxidant activity was determined by the hydroxyl radical scavenging. The results showed that: the total amount of their catechins was between 0.45 (Qingzhuan) ~ 121.21 mg/g (Mengding ganlu), and the order for theirs was green tea > yellow tea > dark tea (black tea); except Qingzhuan, their EGCG contents were between 1.07 ± 0.01 (Chuanhong gongfu) ~ 76.16 ±0.43 mg/g (Mengding ganlu), and the order for theirs was green tea > yellow tea > dark tea (black tea); EGCG3"Me, which only remained in green and yellow tea, their contents were between 0.05±0.02 (Mengding Huangya) ~ 0.39±0.04 mg/g (Mengding ganlu); their hydroxyl radical scavenging was between 48.37 ± 0.20 (Fuzhuan) ~ 75.51 ± 0.63% (Mengding Huangya) and their IC₅₀ was between 3.31 ± 0.028 ~ 5.18 ± 0.012 mg/mL, the order for their clear rates was yellow tea > green tea > dark tea (black tea). Mengding Huangya showed the highest antioxidant activity in Sichuan tea.

1. Introduction

Sichuan is one of the ancient tea areas in China and has been planted tea for more than 3000 years. It mainly produces green tea, yellow tea, black tea and dark tea. Green tea are mainly represented by Zhuyeqing and Mengding ganlu. Yellow tea is Chuanhong gongfu. Black tea is Chuanhong gongfu. Sichuan dark tea originated about eleventh century and mainly divided into the Southern route tea and Western route tea. Southern route tea are divided into Kang brick and Jin jian. Ya’an tibetan tea, suitable for domestic sales, overseas sales and export tea products, is produced based on typical process. Western route Tea is divided into Fang bao and Fuzhuan, but Fuzhuan is replaced by Qingzhuan gradually.¹,² Many biological functions (such as antioxidant) in tea have been reported.³⁻⁵ These beneficial effects are mainly due to tea polyphenols.⁶ Catechins account for about 70-80% of the total tea polyphenols, which are mainly composed of (-) - epicatechin (EC), (-) - epigallocatechin (EGC), (-) - epigallocatechin gallate (EGCG), and (-) - epicatechin gallate (ECG). These catechin isomers (such as EGCG3"Me) may be more active.⁷,⁸ Sichuan tea production have a long history, but catechin components of the Kang brick and Mengdinghuangya were rarely reported,⁹ especially the antioxidant activity of methylated catechin in Sichuan tea was not reported. Based on the above facts, in this study, six catechins (EGC,C,EC, EGCG, ECG,EGCG3"Me) contents of the nine representative
teas (green tea, yellow tea, dark tea, or black tea) produced in Sichuan and their antioxidant activity were systematically analyzed. It provided the basis for the further development and utilization of Sichuan tea.

2. Materials and Methods

2.1. Materials and instruments
Gallic acid, caffeine, C, EC, EGC, EGCG, ECG reference standards (Purchased from Sigma); EGCG3"Me reference standards and Japanese benifuji green tea (By the Key Laboratory of the Tea science, Ministry of Education, Hunan Agricultural University). Samples were collected at random from Sichuan main production enterprises, each sample was collected 10 copies, and the sample was prepared after mixing.

2.2. Instrument and equipment
The DIONEX UltiMate 3000 UHPLC including: DGP-3600SD pump, WPS-3000 AutoSampler, TCC-3000 RS Column, DAD-3000 Detector, AcclaimTM 120 (5 μm, 120 Å, 4.6 mm×250 mm) C18 column, Chromeleon 7 workstation. Velocity 18R centrifuge (Shimadzu, Japan), Arium Comfort purified water machine (Germany Sartorius), UV 2600 UV/Visible Spectrophotometer (Shanghai Tianmei).

2.3. Determination of catechin
2.3.1. Sample extraction. Sample extraction was carried out according to the extraction method of literature[10].

2.3.2. Chromatographic condition. The Catechins contents were determined by referring to the chromatographic conditions of literature[10].

2.4. Determination of hydroxyl radical scavenging activity
Determination of hydroxyl radical scavenging ability in L Wang's method[11] to be improved. Accurately dry tea powder was weighed 250 mg, added 25 mL water, shaked, ultrasonically 10 min, after standing 2 min, filtered. The filter liquor was fixed to 50 mL with water. Blending 1 mL extracting solution, 1 mL FeSO₄·7H₂O solution (9 mmol/L) and 1 mL H₂O₂ (10 mmol/L), 37°C water bath for 10 min, added 1 mL (9 mmol/L), 37°C water bath after 30 min, determinated absorbance of reaction solution in 510 nm, water blank control. Calculation method: hydroxyl radical clearance rate (%) = (1 - sample absorbance/blank group absorbance) x100.

2.5. Data Analysis
All the results were expressed as mean±SD (n=3).

3. Results and Analysis
3.1. Determination of EGCG3"Me content in benifuji green tea
UHPLC chromatogram of catechins, gallic acid and caffeine were shown in Figure 1. From Figure 1, there was a definite peak order: GA, 5.9min; EGC, 21.1 min; CAF, 23.2 min; C, 24.5 min; EC, 36.4 min; EGCG, 37.8 min; EGCG3"Me, 46.7 min; ECG, 50.5 min. At the same time, the Japanese benifuji green tea was determined according to the sample method established in this experiment, and the content of EGCG3"Me was 16.56 mg/g.
3.2. Analysis of catechins in Sichuan Tea

The contents of catechins in Sichuan tea were shown in Table 1, and the elution profiles of some tea were shown in figure 2.

From table 1, the total amount of catechins in the Sichuan tea was between from 0.45 to 121.21 mg/g, the lowest was Kang brick and the highest content was Mengding Ganlu. The order was green tea (117.73 mg/g) > yellow tea (93.48 mg/g) > black tea (8.05 mg/g) and dark tea (6.80 mg/g). The kinds of catechins in green tea and yellow tea contained C, EC, EGC, EGCG, ECG and EGCG3’Me. The contents of catechins in dark tea and black tea were decreased. Only EGC, EGCG, ECG were found in black tea, and only EC, EGC, EGCG, ECG were found in dark tea.
Table 1. The contents of catechins in Sichuan tea (mg/g).

| Sample          | EGC   | C     | EC    | EGCG  | EGCG3"Me | ECG    |
|-----------------|-------|-------|-------|-------|----------|--------|
| Green tea       |       |       |       |       |          |        |
| Mengding ganlu  | 10.23±0.38 | 1.05±0.06 | 7.82±0.27 | 76.16±0.43 | 0.39±0.04 | 25.56±0.08 |
| Zhuyeqing       | 6.81±0.30 | 0.85±0.03 | 8.14±0.29 | 69.92±0.49 | 0.13±0.02 | 27.20±0.09 |
| Yellow tea      |       |       |       |       |          |        |
| Mengding huangya| 7.90±0.69 | 1.05±0.10 | 6.79±0.14 | 53.55±0.44 | 0.05±0.02 | 24.14±0.06 |
| Kangzhuang      | 3.09±0.10 | -      | 1.57±0.05 | 3.43±0.03 | -        | 1.08±0.03 |
| Jinjian         | 1.82±0.14 | -      | 2.62±0.01 | 5.04±0.05 | -        | 1.52±0.05 |
| Dark tea        |       |       |       |       |          |        |
| Ya'an Tibet tea | 1.48±0.13 | -      | 1.07±0.03 | 5.16±0.06 | -        | 1.76±0.03 |
| Fuzhuan         | -      | -      | 0.64±0.03 | 2.44±0.03 | -        | 0.84±0.06 |
| Qingzhuan       | -      | -      | 0.45±0.01 | -      | -        | -       |
| Black tea       |       |       |       |       |          |        |
| Chuanhong gongfu| 3.60±0.16 | -      | -      | 1.07±0.01 | -        | 3.38±0.22 |

"-" means undetected.

The EGCG was not detected in Qingzhuan. The content of EGCG in Sichuan tea was between from 1.07 to 76.16 mg/g and the highest was Mengding ganlu. From 4 tea category, the order for their EGCG contents was green tea (73.04 mg/g) > yellow tea (53.55 mg/g) > dark tea (3.21 mg/g) > black tea (1.07 mg/g).

The EGCG3"Me content was between from 0.05 to 0.39 mg/g. EGCG3"Me was detected only in 3 Sichuan tea, the highest was Mengding ganlu and the lowest was Mengding huangya. From the 4 tea category, EGCG3"Me only in green tea and yellow tea were detected; black tea and dark tea were not detected; green tea (0.26 mg/g) was higher than yellow tea (0.05 mg/g).

3.3 Comparison of scavenging ability of hydroxyl radical in Sichuan tea

From table 2, it can be seen that the scavenging rate of hydroxyl radicals in Sichuan tea was between from 48.37 to 75.51%, and the IC$_{50}$ (concentration of solution at 50% inhibition rate) was between from 3.31 to 5.18 mg/mL. The smaller the IC$_{50}$ value, the greater the antioxidant capacity. Mengding huangya showed the highest antioxidant activity(75.51%), followed by Zhuyeqing(74.84%), Mengding ganlu(73.91%), Ya'an tibetan tea(63.68%), Kang brick (60.13%), Chuanhong gongfu(57.78%), Jinjian (56.77%), Qingzhuan(54.18%), Fuzhuan(48.37%). From the tea category, the order for their clear rates was yellow tea > green tea > dark tea (black tea).

Table 2. Hydroxyl radial scavenging activity of Sichuan tea.

| Sichuan tea     | scavenging activity(%) | IC$_{50}$(mg/mL) |
|-----------------|-------------------------|-----------------|
| Green tea       |                         |                 |
| Mengding ganlu  | 73.91±0.97              | 3.38±0.045      |
| Zhuyeqing       | 74.84±0.28              | 3.34±0.011      |
| Yellow tea      |                         |                 |
| Mengding huangya| 75.51±0.63              | 3.31±0.028      |
| Kangzhuang      | 60.13±0.32              | 4.16±0.016      |
| Jinjian         | 56.77±0.21              | 4.41±0.020      |
| Dark tea        |                         |                 |
| Ya'an Tibet tea | 63.68±1.20              | 3.92±0.078      |
| Fuzhuan         | 48.37±0.20              | 5.18±0.012      |
| Qingzhuan       | 54.18±0.62              | 4.61±0.059      |
| Black tea       |                         |                 |
| Chuanhong gongfu| 57.78±0.26              | 4.33±0.17       |

4. Discussion

In this study, UHPLC was used for the first time to systematically analyze tea catechins mainly in Sichuan green tea, yellow tea, dark tea and black tea. It was found that the order of total catechins contents was in line with EGCG contents: green tea > yellow tea > dark tea (black tea). Only green tea and yellow tea had EGCG3"Me. It was reported firstly that yellow tea had EGCG3"Me. The content of EGCG3"Me in green tea was higher than oolong tea, and black tea, dark tea were not
The results obtained in this study are consistent with previous studies\cite{13,14}. But it is inconsistent with the sequence of antioxidant activity in the tested materials. The main reason is that there is a low linear correlation between hydroxyl radical scavenging activity and EGCG content, and no correlation with the total amount of catechins\cite{15}. Its antioxidant mechanism needs further study.

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