

不同时期大叶苦丁茶(*Ilex latifolia* Thunb.)不同器官中
咖啡酰奎宁酸类成分的毛细管电泳分析

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摘要 大叶苦丁茶(*Ilex latifolia* Thunb.)含有丰富的咖啡酰奎宁酸。在本研究中,运用胶束电动毛细管色谱法,测定了在不同发育时期的大叶苦丁茶不同器官中的咖啡酰奎宁酸含量。大叶苦丁茶的不同器官在不同发育时期的咖啡酰奎宁酸含量有着显著的变化。在叶片中,10天叶龄的叶的咖啡酰奎宁酸含量是19.78 g/100 g,而当叶龄达到12月时,其中的咖啡酰奎宁酸含量已经降为1.41 g/100 g。随着发育时间的增长,雌花、雄花中的含量变化也有类似的变化。雌花(果)和雄花在发育期为10天时的咖啡酰奎宁酸含量高:分别20.29 g/100 g和18.06 g/100 g,随后在雌花发育成的7个月果实中下降到0.16 g/100 g,而在40天期接近脱落的雄花中其含量降为10.36 g/100 g。大叶苦丁茶的器官中,除了嫩叶,雄花由于高含量的咖啡酰奎宁酸和较多的生物量,也是一种好的咖啡酰奎宁酸来源材料。本文首次报道了大叶苦丁茶的花中含有大量的咖啡酰奎宁酸。MEKC方法也是首次被用于苦丁茶中的咖啡酰奎宁酸类成分的测定,使用这种方法,大叶苦丁茶中的6种咖啡酰奎宁酸成分在40分钟内完全分离。相较于以前文献中报道的高效液相色谱法,本文为苦丁茶中的咖啡酰奎宁酸的质量控制提供了一种新的检测方法。

关键词 咖啡酰奎宁酸衍生物;大叶苦丁茶(*Ilex latifolia* Thunb.);MEKC;年龄;花;叶片

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Caffeoylquinic Acid Derivatives in DAYE Kudingcha(*Ilex latifolia* Thunb.)
Organs in Differently Developing Stage by Micellar
Electrokinetic Chromatography

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Abstract DAYE Kudingcha is rich in caffeoylquinic acid derivatives(CQAs). CQAs in DAYE Kudingcha organs in different developing stages were determined by micellar electrokinetic chromatography(MEKC). The composition of CQAs varied greatly in tissues at differently developing stages. The content of CQAs was 19.78 g/100 g in leaves at the age of 10 days, and then decreased to 1.41 g/100 g at 12 months. A similar predisposition was observed in female and male flowers with the increasing age. Female flowers(fruits) and male flowers at the age of 10 days have a high content of CQAs : 20.29 g/100 g and 18.06 g/100 g, respectively, which subsequently decreased to 0.16 g/100 g in 7-month mature fruits, 10.36 g/100 g in 40 day falling male flowers. The male flowers, as well as the young leaves, might be a good CQAs' raw material, due to a large amount of CQAs and biomass of the male flowers in the Kudingcha species. This is the first report describing the contents of CQAs in the flowers of Kudingcha. MEKC method was also used for the first time in the determination of CQAs in Kudingcha. Compared to the HPLC method described previously, the MEKC might be an alternative for the quality control of CQAs in Kudingcha.

Key words caffeoylquinic acid derivatives; *Ilex latifolia* Thunb.; MEKC; age; flowers; leaves

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Ilex latifolia Thunb. , a bitter tea of Chinese origin , is widely consumed in East Asia and is commonly known as DAYE Kudingcha in Chinese. In traditional Chinese Medicine , it was prescribed for the treatment of clearing the heat , dispelling the wind , quenching the thirst , and headache^[1]. Recently , *I. latifolia* is reported to possess several beneficial functions , such as antioxidant , antiobesity , antidiabetic , anti-inflammatory , and hepatoprotective effects^[2~3].

Various pharmacological effects and properties inherent to *I. latifolia* and other plants have been attributed to their phenolic composition^[4~7]. The main classes of phenolic compounds found in the large leaf Kudingcha , are caffeoylquinic acid derivatives (CQAs)^[8~9] that consist of chlorogenic acid (3-CQA) and other five chlorogenic acid derivatives , including 4-CQA , 5-CQA , 3 , 5-diCQA , 4 , 5-diCQA , and 3 , 4-diCQA. The Kudingcha originating from *I. latifolia* might be classified as the chlorogenic acid teas due to its large amount of CQAs as compared to the green tea , which contains abundant catechins^[8].

According to a recent study , the contents of CQAs varied greatly in Kudingcha leaves. Previously , Negishi et al^[8] reported that Kudingcha contained a large amount of CQAs. Also , great variations in the content of CQAs were observed with respect to different Kudingcha leaves ' samples , i. e. 3 , 5-diCQA (0. 54% - 10. 6% , dry weight ; dw). Midorikawa et al^[9] compared the CQAs between the spring and autumn leaves in this Kudingcha species and found a considerable variability in their amounts. Historically , the leaves of *I. latifolia* were the organ for preparing the Kudingcha beverage^[10]. In this plant , leaves are usually born in the first to the third year branchlets , while a large number of inflorescences are born in the axil of biennial branches^[11]. For better utilization of this plant resources , it is necessary to carry out investigations in the CQAs content of the flowers , and more detailed data on the leaves are also needful.

HPLC , as a primary analytical method , has been applied to analyze the chlorogenic acid in the leaves of Kudingcha and other *Ilex* species^[8~9, 12~13]

However , HPLC used in the analysis of traditional Chinese medicines exhibit some limitations , including low-resolution and short column lifetime owing to easy contamination^[14~15]. Capillary electrophoresis (CE) with UV approaches have not yet been used for this purpose. Despite the weakness in repeatability , capillary electrophoresis is recognized as a critical analytical separation technique because of its speed , efficiency , ultra-small sample volume , little consumption of the solvent , and simple cleaning-up.

In this study , an isolation method for the six derivatives of CQA in *I. latifolia* was established using Micellar electrokinetic chromatography (MEKC). The six derivatives of CQA were analyzed in different organs (leaves , female/male flowers) of *I. latifolia* in different developing stages. The contents of roots and stems 1-year-old were also investigated. To the best of our knowledge , this is the first report describing the CQAs in sexual organs of *I. latifolia*. Our findings stated that both the male flowers and young leaves have high amounts of CQAs and the biomass of the male flowers was richer than young leaves in *I. latifolia*. Thus , this characteristic might provide an alternative resource of CQAs in the plant.

1 Materials and methods

1.1 Chemicals

Neochlorogenic acid was purchased from Sigma-Aldrich (St. Louis , MI , USA). 3-CQA , 4-CQA , 3 , 5-diCQA , 4 , 5-diCQA , and 3 , 4-diCQA were purchased from Chengdu Mann-Stewart Biological Technology Co. Ltd (Chengdu , China). Sodium dodecyl sulfate (SDS) was obtained from Sigma-Aldrich ; whereas , sodium tetraborate , boric acid , and methanol were from Hao En Biotech (Shanghai , China). HPLC-grade water was obtained from a Milli-Q system (Millipore , Billerica , MA , USA) , and sodium dihydrogen phosphate dehydrate and β -cyclodextrin from SCRC. Ltd. (Shanghai , China)

1.2 Plant material

I. latifolia grow in the Fujian Institute of Subtropical Botany (altitude 118°04'04" , latitude 24°26'46" , annual average temperature 22℃). The samples were collected in 2014 and 2015. Young leaves

and young buds began to develop in late February. Flowers bloomed in March-April , and fruits matured in August-September. The axillary buds grow up to 3 cm , purple/green , and were named as the 10-d-stage. Fourteen samples of leaves were collected at the 10-d- , 20-d- , 30-d- , 2-month- , 3-month- , up to , 12-month-stages. The blooming period of the male flowers was approximately 40 d , and the samples collected were 10- , 20- , 30- , and 40-day-flowers. The blooming period of the female flowers was about 7 months , and the samples collected were 10- , 20- , 30-day-flowers , 2-month-flowers(fruits) , up to 7-month-fruits. The roots and stems were old in March , in which , the axillary buds occurred. All above samples were collected , dried at 50℃ , grilled , and stored at -30℃.

1.3 Sample preparation

The 0.2 g of each sample was solubilized in 8 mL methanol. The samples were stored for 14 h at 4℃ between two ultrasonic extractions for 50 min at 50℃. Finally , the samples were cooled to room temperature and filtered through a 0.45 μm RC filter (SRI , Roth , Germany) prior to a 15 s injection on MEKC.

1.4 Apparatus and MEKC procedures

The CL1030 capillary electrophoresis instrument was equipped with a multi-wavelength UV-Vis scanning detector(Cailu Scientific Instruments Co. , Ltd , Beijing , China). The separations were achieved in a plain fused-silica capillary with 45 cm effective length to the detector. Data processing was performed using an HW-2000 chromatographic working station software version 2.21. The samples were injected by fluid pressure(1 013 Pa). An injection time of 15 s was used for all the analyses. UV absorption was monitored at 326 nm. The separation voltage was 15 kV at a temperature of (26 ± 0.2)℃. The separation buffer(pH of 6.2) consisted of 80 mmol · L⁻¹ sodium dihydrogen phosphate dehydrate , 15 mmol · L⁻¹ SDS , 40 mmol · L⁻¹ sodium tetraborate , 5 mmol · L⁻¹ β-cyclodextrin , and 15% (v/v) methanol. Before the injection of each sample , the capillary was pre-washed for 5 min with the separation buffer.

2 Results and discussion

2.1 Analytical quality control

Under the optimal assay conditions , a mixture of standards solution containing six derivatives of CQA (3-CQA , 4-CQA , 5-CQA , 3 , 5-diCQA , 4 , 5-diCQA , and 3 , 4-diCQA) was used to establish the standard curve ranging from 5 - 250 μg · mL⁻¹. A linear correlation between the peak area and concentration was found in this range for the six analytes (Table 1). The limits of detection were determined from the average standard deviation of triplicate samples with six analytes. The detection limits of the six analytes were as follows : 3-CQA 0.74 μg · mL⁻¹ , 4-CQA 0.87 μg · mL⁻¹ , 5-CQA 1.17 μg · mL⁻¹ , 3 , 5-diCQA 1.33 μg · mL⁻¹ , 4 , 5-diCQA 1.09 μg · mL⁻¹ and 3 , 4-diCQA 1.89 μg · mL⁻¹(Table 1).

Table 1 Regression analysis of calibration curves and detection limits

CQA derivatives	Regression equation	Correlation coefficient (%)	Linear range (μg · mL ⁻¹)	Detection limit (μg · mL ⁻¹) (S/N = 3)
3-CQA	y = 6.844 1e - 003x - 5.312 3	0.9943	5 - 250	0.74
4-CQA	y = 7.087 8e - 003x + 5.369 3	0.9991	5 - 250	0.87
5-CQA	y = 6.640 9e - 003x + 0.484 8	0.9977	5 - 250	1.17
3 , 5-diCQA	y = 6.248 5e - 003x - 1.224 4	0.9987	5 - 250	1.33
4 , 5-diCQA	y = 6.038 1e - 003x - 0.991 3	0.9992	5 - 250	1.09
3 , 4-diCQA	y = 6.576 6e - 003x + 4.301 6	0.9947	5 - 250	1.89

The reproducibility was investigated by a repetitive injection of a 100 μg · mL⁻¹ standard mixture solution under the selected optimum conditions. The relative standard deviations(RSD) of the peak area were 2.69 , 3.09 , 4.96 , 4.26 , 3.77 , and 4.61% for 3-CQA , 4-CQA , 5-CQA , 3 , 5-diCQA , 4 , 5-diCQA and 3 , 4-diCQA , respectively(n = 6).

The recovery was determined as 97.94% - 121.74% by standard addition method with 30 d male flowers ' sample to further evaluate the precision and accuracy of the method(n = 3). The result indicated that the method was accurate for the determination of the above analytes.

In previous studies on the different CQAs with HPLC method , the CQAs were stable in the acidic

mobile phase. The quantification of one CQA derivative was accomplished by comparing the area of the UV peak with that of the standard. The quantification of the other CQA derivatives was performed with reference to the above standard , combined with molar extinction coefficients , and using the following equation^[16 ~ 17] :

$$C = \frac{RF\varepsilon_1MR_2A}{\varepsilon_2MR_1}$$

As the separation of the CQAs in MEKC is based on an entirely different principle to that in HPLC , it is worth investigating the possibility of the usage of one CQA derivative standard to estimate the contents of the other CQA derivatives. In this MEKC method , we used all the six CQAs standard samples and obtained six independent linear equations(Table 1). Using the molar extinction coefficients equation

as described above , and one of the CQAs as the only standard , the concentrations corresponding to the peak areas of other CQA derivatives could be calculated respectively. Compared to the concentration of the standards , the relative differences were within 10% . Thus , it could be deduced that various CQAs in the capillary electrophoresis exhibited similar stability. This feature can be optimally used for estimating the content of the six CQA derivatives by the molar extinction coefficient equation in the MEKC method.

2.2 Quantification of CQAs

Six CQA derivatives , 3-CQA , 4-CQA , 5-CQA , 3 ,5-diCQA , 4 ,5-diCQA , and 3 ,4-diCQA , in the Kudingcha were separated by MEKC in 40 min , as shown in Fig. 1. Table 2 listed the contents of the CQAs from the leaves , male flowers , and female flowers in different stages.

Table 2 Content of CQAs in Kudingcha(<i>I. latifolia</i> Thunb.) organs at differently developing stage ^a								
Organ	Age	3-CQA ^b	4-CQA ^b	5-CQA ^b	3 ,5-diCQA ^b	4 ,5-diCQA ^b	3 ,4-diCQA ^b	total CQAs ^b
Leaves	10 days	3.82 ± 1.36	0.33 ± 0.06	1.36 ± 0.76	12.79 ± 1.92	1.31 ± 0.84	0.16 ± 0.09	19.78 ± 2.77
	20 days	3.36 ± 0.51	0.43 ± 0.10	1.59 ± 0.87	12.16 ± 1.59	1.57 ± 0.31	0.07 ± 0.04	19.19 ± 0.27
	30 days	1.43 ± 0.47	0.42 ± 0.18	0.81 ± 0.30	3.64 ± 1.00	1.14 ± 0.38	0.10 ± 0.04	7.56 ± 1.43
	2 months	1.65 ± 0.33	0.57 ± 0.19	1.19 ± 0.10	1.21 ± 0.16	0.51 ± 0.25	0.05 ± 0.02	5.18 ± 0.25
	3 months	1.82 ± 0.65	0.54 ± 0.20	0.59 ± 0.16	0.75 ± 0.08	0.28 ± 0.06	0.06 ± 0.03	4.04 ± 1.06
	4 months	1.69 ± 0.38	0.56 ± 0.04	0.69 ± 0.16	0.63 ± 0.03	0.24 ± 0.02	0.09 ± 0.03	3.91 ± 0.26
	5 months	1.68 ± 0.15	0.47 ± 0.06	0.72 ± 0.22	0.65 ± 0.30	0.25 ± 0.02	0.03 ± 0	3.81 ± 0.42
	6 months	1.42 ± 0.65	0.48 ± 0.16	0.40 ± 0.19	0.31 ± 0.16	0.21 ± 0.05	0.07 ± 0.04	2.89 ± 1.20
	7 months	1.06 ± 0.16	0.39 ± 0.09	0.35 ± 0.19	0.29 ± 0.11	0.33 ± 0.17	0.06 ± 0.04	2.48 ± 0.08
	8 months	1.06 ± 0.43	0.44 ± 0.13	0.28 ± 0.12	0.12 ± 0.06	0.14 ± 0.02	0.03 ± 0	2.07 ± 0.76
	9 months	0.73 ± 0.28	0.33 ± 0.05	0.15 ± 0.10	0.08 ± 0.04	0.20 ± 0.03	0.03 ± 0	1.53 ± 0.20
	10 months	0.82 ± 0.37	0.22 ± 0.06	0.12 ± 0.03	0.09 ± 0.03	0.20 ± 0.07	0.03 ± 0	1.48 ± 0.46
Female Flowers (fruits)	11 months	0.68 ± 0.37	0.27 ± 0.05	0.08 ± 0.05	0.06 ± 0.03	0.15 ± 0.05	0.03 ± 0	1.27 ± 0.55
	12 months	0.71 ± 0.21	0.40 ± 0.11	0.24 ± 0.11	0.01 ± 0.01	0.03 ± 0.02	Nd ^c	1.41 ± 0.48
	10 days	3.57 ± 0.57	0.39 ± 0.03	1.98 ± 0.66	12.29 ± 2.89	1.93 ± 0.16	0.14 ± 0.07	20.29 ± 3.97
	20 days	1.29 ± 0.18	0.50 ± 0.09	2.20 ± 0.23	11.61 ± 0.03	1.68 ± 0.68	0.20 ± 0.03	17.49 ± 1.85
	30 days	1.31 ± 0.38	0.30 ± 0.15	1.20 ± 0.40	6.67 ± 2.06	1.26 ± 0.41	0.14 ± 0.05	10.88 ± 3.08
	2 months	1.74 ± 0.43	0.49 ± 0.12	1.37 ± 0.35	3.20 ± 0.48	0.89 ± 0.21	0.03 ± 0	7.72 ± 0.88
	3 months	0.66 ± 0.37	0.30 ± 0.13	0.41 ± 0.19	0.76 ± 0.36	0.28 ± 0.11	0.03 ± 0.01	2.44 ± 1.12
	4 months	0.24 ± 0.09	0.14 ± 0.01	0.12 ± 0.07	0.25 ± 0.08	0.08 ± 0.06	0.03 ± 0.01	0.86 ± 0.29
	5 months	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	Nd ^c	Nd ^c	0.12 ± 0.05
	6 months	0.02 ± 0.01	0.06 ± 0.01	0.01 ± 0.01	0.01 ± 0	Nd ^c	Nd ^c	0.12 ± 0.02
Male flowers	7 months	0.02 ± 0	0.08 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	Nd ^c	Nd ^c	0.16 ± 0.04
	10 days	2.71 ± 0.35	0.23 ± 0.12	1.87 ± 0.61	11.77 ± 1.79	1.38 ± 0.51	0.09 ± 0.04	18.06 ± 3.31
	20 days	2.12 ± 0.45	0.31 ± 0.10	1.59 ± 0.47	9.79 ± 1.16	1.14 ± 0.41	0.22 ± 0.03	17.15 ± 1.92
	30 days	1.66 ± 0.16	0.27 ± 0.14	1.53 ± 0.26	7.19 ± 0.68	1.51 ± 0.82	0.11 ± 0.07	12.29 ± 2.10
	40 days	1.17 ± 0.14	0.18 ± 0.03	1.27 ± 0.10	6.34 ± 0.97	1.23 ± 0.22	0.17 ± 0.08	10.36 ± 1.38
Stems	1 year	0.33 ± 0.24	0.20 ± 0.14	0.04 ± 0.01	0.01 ± 0.01	0.03 ± 0.03	0.02 ± 0	0.63 ± 0.29
Roots	1 year	0.07 ± 0.03	0.06 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.06 ± 0.04	0.04 ± 0.02	0.28 ± 0.20

Note :^a Results are shown as the means of extractions in duplicates ± standard deviation , expressed in g/100 g of dry weight organs ;^b CQA = caffeoylquinic acid ; diCQA = dicaffeoylquinic acid ; total CQAs matched six caffeoylquinic acid derivatives ;^c Nd = not detected

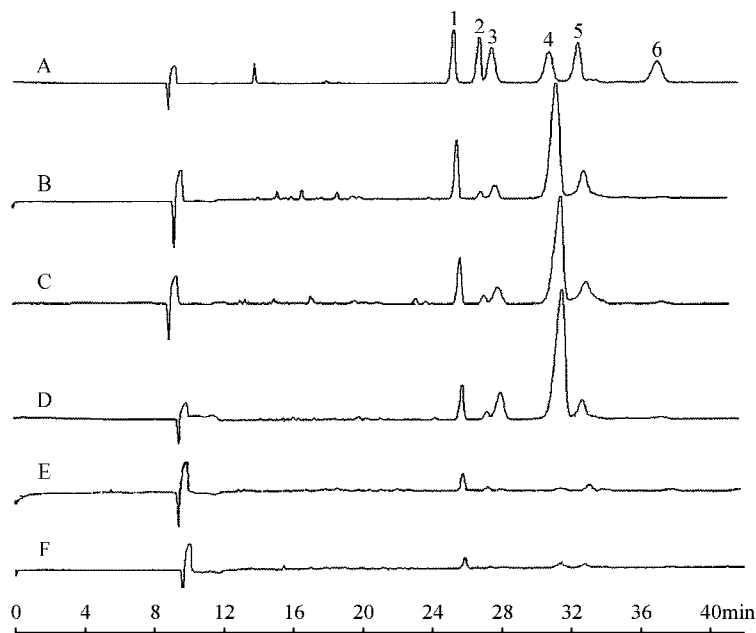


Fig.1 Electropherogram of standards mixture and part samples A. Six caffeoylquinic acid derivatives standards mixture ; B. Leaves of 20 d ;C. Female flowers of 20 d ;D. Male flower of 20 d ;E. Stems of one year ;F. Roots of one year Peaks :1. 3-CQA ;2. 4-CQA ;3. 5-CQA ;4. 3 5-diCQA ;5. 4 5-diCQA ;6. 3 4-diCQA

The young organs(leaves and flowers) ≤ 20 d have high amounts of CQAs ranging from 17. 15 – 20. 29 g/100 g(dw). However , the contents decreased with increasing age. The content of the CQAs was 19. 78 g/100 g(dw) , and that of 3 5-diCQA was the highest , amounting to 12. 79 g/100 g(dw) in 10-day-age leaves. 3 5-diCQA was useful in anti-gastric cancer^[18]. The contents of the derivatives were stable in 20-day-age leaves and decreased to 7. 56 g/100 g(dw) in 30 d leaves. Additionally , the content of 3 5-diCQA decreased to 3. 64 g/100 g(dw). This phenomenon might be attributed to the decreasing synthesis of CQAs and increasing leaves that led to a decline in the relative contents. The trend continued to the 12-month leaves , in which , the content of the total CQAs was 1.41 g/100 g(dw).

Similar results were obtained in flowers and fruits of *I. latifolia*. During 40 days in blooming for the male flowers , the contents of the CQAs decreased with growth development. The content of the derivatives was 18. 06 g/100 g(dw) and 10. 36 g/100 g(dw) at the 10-d- and 40-d-stages , respectively. On the other hand , the total content of the CQAs was 20. 29 g/100 g(dw) in the 10 d female flowers and

decreased to 0. 16 g/100 g(dw) in the 7-month female fruits ;less content was detected in 1-year roots and stems.

In young leaves and flowers of *I. latifolia* , 3 5-diCQA content accounted for about 60% of total CQAs. The predominance of 3 5-diCQA and Proportions of other CQAs were in accordance with reports from Midorikawa et al^[9]. Yerba mat(*Ilex paraguayensis*) , a species belonging to the same genus as Kudingcha , also contained a large amount of CQAs. Interestingly , there were no difference in the CQAs content found between the young(1 month) and mature(6 months) leaves^[19]. The metabolism of plant components is complex , The genetics-species , the type of soil , the age of the tissue , and exposition to light or shadow is known to influence the amount of bioactive substances in *Ilex* species^[20 ~ 21]. The contents of CQAs varied sharply in different organs of Kudingcha with changing age. It might be deduce that the age was the main factor influencing the content of the CQAs in this Kudingcha species.

Conventionally , the leaves of Kudingcha were used as beverage materials. Based on our study , the male flowers of Kudingcha were potentially better ma-

terials for this health-friendly chlorogenic acid teas. *I. latifolia* is a dioecious plant. In the current investigation, the terminal buds were differentiated into leaf buds in spring, while the axillary buds were usually differentiated into flower buds(Fig. 2). In female plants, the number of flower buds was slightly more

than the buds, and in the male plants, the number of flower buds was much more than the leaf buds. The average weight of single 20 d male inflorescence was 0.26 g(dw), which was similar to that of the leaf buds. Therefore, the male flowers might prove to be a better alternative resource of CQAs for this plant.

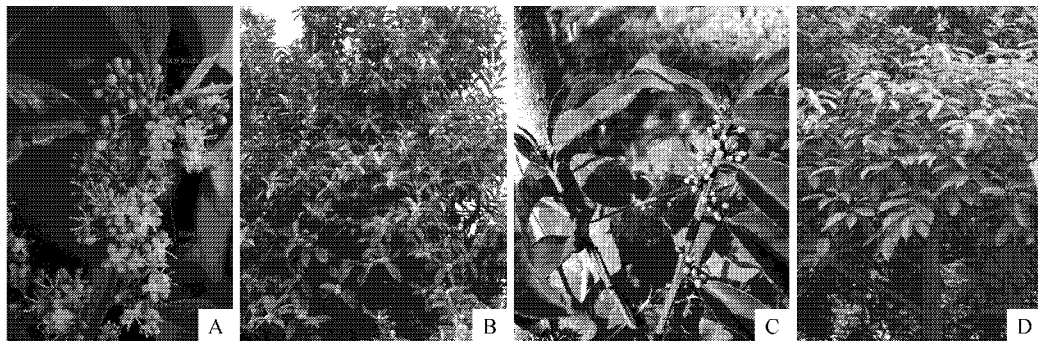


Fig. 2 Male inflorescences female inflorescences and leaf buds in *I. latifolia*

A. Male flower branch ; B. Male tree ; C. female flower branch ; D. female tree

3 Conclusions

Several investigations on Kudingcha compounds were carried out previously using HPLC. An MEKC method was applied to analyze the six CQAs in Kudingcha for the first time; the six compounds could be separated within 40 min. Thus, MEKC might be an alternative for the quality control of the CQAs in Kudingcha species.

As compared to the previous study, we investigated the CQAs in Kudingcha organs including leaves, female flowers(fruits), and male flowers at different ages. The roots and stems of 1-year-old were also investigated. The different young organs within 20 d had similar high amounts of CQAs, ranging from 17.15 – 20.29 g/100 g(dw). The amounts of CQAs in Kudingcha flowers were reported for the first time, and the biomass of the male flowers was higher than the young leaves, a traditional organ for the preparation of the Kudingcha beverage. Our survey indicated that the male flowers might be a better resource of CQAs for this plant.

Age might be the main factor influencing the content of the CQAs. The content of CQAs varied greatly in different organs of Kudingcha with changing age. This information was helpful for selecting the

most adequate Kudingcha fraction as the raw material for the preparation of CQAs-enriched extractions.

参 考 文 献

1. 谢观, 樊正伦, 张年顺. 中国医学大辞典[M]. 北京: 中国中医药出版社, 1994.
Xie G, Fan Z L, Zhang L X. Grand dictionary of Chinese medicine[M]. Beijing: Beijing Traditional Chinese Medicine Press, 1994.
2. Hu T, He X W, Jiang J G. Functional analyses on antioxidant, anti-inflammatory, and antiproliferative effects of extracts and compounds from *Ilex latifolia* Thunb. a Chinese bitter tea[J]. Journal of Agricultural and Food Chemistry, 2014, 62(34) : 8608 – 8615.
3. Hu T, He X W, Jiang J G, et al. Efficacy evaluation of a Chinese bitter tea(*Ilex latifolia* Thunb.) via analyses of its main components[J]. Food & Function, 2014, 5(5) : 376 – 381.
4. Shahrzad S, Bitsch I. Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography[J]. Journal of Chromatography A, 1996, 741(2) : 223 – 231.
5. Farah A, De Paulis T, Moreira D P, et al. Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees[J]. Journal of Agricultural and Food Chemistry, 2006, 54(2) : 374 – 381.
6. Thuong P T, Su N D, Ngoc T M, et al. Antioxidant activity and principles of Vietnam bitter tea *Ilex kudingcha*[J].

- Food Chemistry 2009 ,113(1) :139 – 145.
7. Song C W ,Xie C ,Zhou Z W ,et al. Antidiabetic effect of an active components group from *Ilex kudingcha* and its chemical composition[J]. Evidence-Based Complementary and Alternative Medicine 2012 ,2012 :423690.
8. Negishi O ,Negishi Y ,Yamaguchi F ,et al. Deodorization with ku-ding-cha containing a large amount of caffeoyl quinic acid derivatives[J]. Journal of Agricultural and Food Chemistry 2004 ,52(17) :5513 – 5518.
9. Midorikawa M ,Ohnishi-Kameyama M ,Nagata T. Seasonal difference of caffeic acid derivative contents in current-year leaves and old leaves of *Ilex latifolia* Thunb.[J]. Journal for the Integrated Study of Dietary Habits 2010 ,20(4) :305 – 312.
10. 江苏新医学院. 中药大辞典[M]. 上海 :上海科学技术出版社 ,1985.
- Jiangsu College of New Medicine. Dictionary of Chinese medicine[M]. Shanghai :Shanghai Press of Science and Technology ,1985.
11. 中国科学院中国植物志编辑委员会. 中国植物志[M]. 北京 :科学出版社 ,1999 :107 – 110.
- Academicae Sinicae Edita. Flora of China[M]. Beijing : Science Press ,1999 :107 – 110.
12. Marques V ,Farah A. Chlorogenic acids and related compounds in medicinal plants and infusions[J]. Food Chemistry 2009 ,113(4) :1370 – 1376.
13. Liu L X ,Sun Y ,Laura T ,et al. Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C. J. Tseng[J]. Food Chemistry , 2009 ,112(1) :35 – 41.
14. Chu Q C ,Lin M ,Tian X H ,et al. Study on capillary electrophoresis-amperometric detection profiles of different parts of *Morus alba* L.[J]. Journal of Chromatography A , 2006 ,1116(1 – 2) :286 – 290.
15. 徐夙侠 林春松 黄青云 ,等. 3 个三角梅品种中类黄酮的毛细管电泳分析比较研究[J]. 植物研究 ,2010 ,30(6) :718 – 724.
- Xu S X ,Lin C S ,Huang Q Y ,et al. Analysis and comparison of flavonoids in three bougainvilleas by Micellar Electrokinetic Chromatography[J]. Bulletin of Botanical Research 2010 ,30(6) :718 – 724.
16. De Paulis T ,Commers P ,Farah A ,et al. 4-Caffeoyl-1,5-quinide in roasted coffee inhibits[³H] naloxone binding and reverses anti-nociceptive effects of morphine in mice [J]. Psychopharmacology 2004 ,176(2) :146 – 153.
17. Farah A ,De Paulis T ,Trugo L C ,et al. Effect of roasting on the formation of chlorogenic acid lactones in coffee[J]. Journal of Agricultural and Food Chemistry 2005 ,53(5) :1505 – 1513.
18. 王明彦. 苦丁茶中二咖啡酰基奎宁酸对人肿瘤细胞的体外抑制作用及其机制研究[D]. 武汉 :湖北中医药大学 2014.
- Wang M Y. The inhibitory effects of dicaffeoylquinic acid from *Ilex kudingcha* on human cancer cells and its mechanism of action in vitro[D]. Wuhan :Hubei University of Chinese Medicine 2014.
19. Dartora N ,De Souza L M ,Santana-Filho A P ,et al. UPLC-PDA-MS evaluation of bioactive compounds from leaves of *Ilex paraguariensis* with different growth conditions ,treatments and ageing[J]. Food Chemistry ,2011 ,129(4) :1453 – 1461.
20. Farag M A ,Porzel A ,Wessjohann L A. Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS ,LC-MS and 1D NMR techniques[J]. Phytochemistry 2012 ,76 :60 – 72.
21. Butiuk A P ,Martos M A ,Adachi O ,et al. Study of the chlorogenic acid content in yerba mate(*Ilex paraguariensis* St. Hil.) Effect of plant fraction ,processing step and harvesting season[J]. Journal of Applied Research on Medicinal and Aromatic Plants 2016 ,3(1) :27 – 33.