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# Comparison of chemical compositions of *Ilex latifolia* Thumb and *Camellia sinensis* L.

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**Abstract**

Chemical composition of two beverage plant species, i.e. *Ilex latifolia* and *Camellia sinensis* were investigated. Fifteen and sixteen amino acids were detected in *I. latifiolia* and *C. sinensis,* respectively. Major amino acids were histidine, aspartic acid and glutamic acid in *I. latifolia* but theanine, glutamic acid and histidin in *C. sinensis*. Ascorbic acid and polyphenols in *I. latifiolia* were 0.46 mg $g^{-1}$ and 90.1 mg $g^{-1}$, less than one fourth and one half of those in *C. sinensis,* respectively. Eight catechin compounds were found in *C. sinensis* but only four in *I. latifolia*, i.e. L-catechin (C), L-epicatechin (EC), L-epicatechin gallate (ECG) and L-catechin gallate (CG). Three flavonoids, i.e. rutin, mericetin and quercetin were detected and their total contents in *I. latifolia* and *C. sinensis* were 4781 mg $kg^{-1}$ and 580 mg $kg^{-1}$, respectively. Caffeine was not detected in *I. latifolia*. The higher concentration of flavonoids in leaf of *I. latifolia* may be related to its value for a healthy beverage. 

*Keywords:* *Ilex latifolia*; *Camellia sinensis*; Amino acids; Polyphenols; Caffeine

## 1. Introduction

Extract of leaf from *Ilex Latifolia* Thumb, which is said to aid weight loss, as well as quenching thirst, has been used as a tea leaf beverage (*Cameilio sinensis* L.) in China and Southeast Asia (He, 2000; Li & Li, 1996; Liu, Liang & Xu, 1991). It is also called *Kuding Cha*, i.e. bitter tea by the Chinese because it tastes much more bitter than tea (*C. sinensis* L). Little information about the chemical composition of *I. latifolia* has been reported. The present work is set out to compare the contents of amino acids, polyphenols, ascorbic acid, caffeine and some flavonoids of the leaves of the two plant species.

## 2. Materials and methods

### 2.1. Materials

Leaf sample of *I. latifolia* Thumb, which were sold as beverages, were produced by the Xinchang Tea Company of Zhejiang Province and a sample of green tea was processed from the leaf of *C. sinensis* var. Yunnandaye by Fenghe Sci-Tech Ltd Co., Chongqing, China. The samples were ground and sieved (60 meshes per inch).

HLPC reference compunds were provided by Dr. Takeda from the National Institute of Vegetables, Ornamental Plants and Tea of Japan. Acetonitrile, methanol and acetic acid were HLPC reagents, produced by Tianjin Shild Biometric Technical Co., Ltd., China.

### 2.2. HPLC analysis of amino acids

The ground sample (0.5 g) was placed in a glass flask with 75 ml boiling distilled water and extracted for 45 min on a boiling water bath and then left at room temperature. The resultant liquor was filtered through "Double-ring" No. 102 filter paper (produced by Xinhua Papermaking Ltd Co., Hangzhou, China) and the residue was discarded. The filtrate was dried in a vacuum evaporator to produce a dry powder. The powder was dissolved in 6 ml of solution consisting of 0.1 M HCl and methanol (70:30, v). The resulting solution was purified through a $C_{18}$ column (4.6×250 mm) and 0.45 μm Millipore filter. The filtrate (20 μl) was blended with reagent (100 μl) and then was injected into the HLPC. The reagent consisted of 80 mg 1,2-dialdehygrobenzene (previoulsy dissolved in 1 ml methanol) and 60 μg β-mercaptoethanol dissolved in 1 ml of 0.4

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mol sodium borate buffer (ph9.5). The chromatographic conditions were as follows:

| | |
|---|---|
| Injection volume: | 10 μl |
| Column: | Amino acid analysis column |
| Column temperature: | 62 °C |
| Mobile phase: | Solvent A: 0.2 M sodium citrate buffer (pH 3.0); Solvent B: 0.2 M sodium borate buffer (pH 9.6) |
| Gradient: | 100% solvent A to solvent A: solvent B (48:52, V) by linear gradient during 90 min |
| Flow rate: | 0.4 ml $min^{-1}$ |
| Detector: | Beckman model-157 fluorimetric detector, excitation 338 nm, emission 425 nm filters |
| Sensitivity: | 0.10 aufs |

### 2.3. Analysis of caffeine and catechin compounds by HPLC

Sample (3 g) was extracted in a conical flask containing 250 ml boiling distilled water on a boiling water bath for 30 min. The resultant liquor was filtered though "Double-ring" No. 102 filter paper when it was cooled to room temperature. The filtrate was then filtered through a 0.45 μm Millipore filter. The final filtrate was used for HPLC analysis. The chromatographic conditions were as follows:

| | |
|---|---|
| Injection volume: | 10 μl |
| Column: | 5μ-Diamonsil™ $C_{18}$, 4.6 ×250 mm |
| Column temperature: | 38 °C |
| Mobile phase: | Solvent A: acetonitrile/ acetic acid/water (6:1:193, v:v:v); |
| Solvent B: | acetonitrile/acetic acid/water (60:1:139, v:v:v) |
| Gradient: | 100% (v) solvent A to 100% (v) solvent B by linear gradient during the first 45 min and then 100% (v) solvent B from 45 min until 60 min. |
| Flow rate: | 1 ml $min^{-1}$ |
| Detector: | Shimadzu SPD ultraviolet detector, 280 nm |
| Sensitivity: | 0.01 aufs |

### 2.4. Analysis of ascorbic acid by HPLC

The ground sample (0.5 g) was macerated in a glass blender containing 5 ml of metaphosphate solution (3 g $l^{-1}$). The macerate was transferred into a graduated flask and diluted to 25 ml with distilled water. The diluted solution was filtered through "Double-ring" No.102 filter paper and then a 0.45 μm Millipore filter. The filtrate was injected into HPLC. The HPLC conditions were as follows:

| | |
|---|---|
| Injection volume: | 10 μl |
| Column: | μ-Bondapak $C_{18}$, 3.9×150 mm |
| Column temperature: | 30 °C |
| Mobile phase: | $(NH_4)H_2PO_4$ water solution (200 g $l^{-1}$, pH2.8) |
| Flow rate: | 1 ml $min^{-1}$ |
| Detector: | Shimadzu SPD ultraviolet detector, 254 nm |
| Sensitivity: | 0.10 aufs |

### 2.5. Analysis of flavonoids by HPLC

The ground sample (10 g) was soaked in a flask containing 50 ml petroleum ether for over 12 h to remove the pigments. The solvent was filtered through "Double-ring" No.102 filter paper and discarded. The residue was air dried and was then extracted in a glass flask containing 80 ml ethanol solution (850 ml $l^{-1}$) under a reflux condenser at 80 °C for 1 h. The extract was filtered through "Double-ring" No.102 filter paper and the residue was re-extracted twice more as above. The three filtrates were blended and concentrated under vacuum to approximately 100 ml. An aliquot (5 ml) of the concentrate was filtered through a Sep-Pak column to remove its pigments and particles. The final filtrate was injected into HPLC. The HPLC conditions were as follows:

| | |
|---|---|
| Injection volume: | 2 μl |
| Column: | μ-Bondapak fatty acid column, 4×300 mm |
| Column temperature: | 30 °C |
| Mobile phase: | methanol solution (550 ml $l^{-1}$, pH3.0) |
| Flow rate: | 1.2 ml $min^{-1}$ |
| Detector: | Shimadzu SPD ultraviolet detector, 254 nm |
| Sensitivity: | 0.10 aufs |

Table 1
Comparison of amino acid compositions between *Ilex latifolia* and *Camellia sinensis*

| Amino acids | *I. latifolia* (mg kg$^{-1}$) | *C. sinensis* (mg kg$^{-1}$) |
|---|---|---|
| Aspartic acid | 256 | 1589 |
| Threonine | 140 | 1184 |
| Serine | 84.3 | 1227 |
| Theanine | 0 | 11794 |
| Glutamic acid | 246 | 5094 |
| Glycine | 3.5 | 486 |
| Alanine | 66.1 | 684 |
| Valine | 8.4 | 36.8 |
| Isoleucine | 15.8 | 152 |
| Leucine | 34.6 | 244 |
| Tyrosine | 0.3 | 216 |
| Phenylalanine | 0.2 | 188 |
| Histidine | 1112 | 3653 |
| Tryptophan | 0.5 | 0.7 |
| Lysine | 1.3 | 27.2 |
| Arginine | 0.8 | 4482 |
| Total | 1969 | 31058 |

Table 2
Comparison of caffeine, ascorbic acid and polyphenols between *Ilex latifolia* and *Camellia sinensis* (mg g$^{-1}$)

| Samples | Ascorbic acid | Polyphenols | Caffeine |
|---|---|---|---|
| *I. latifolia* | 0.46 | 90.1 | 0 |
| *C. sinensis* | 2.08 | 187 | 49.5 |

Table 3
Composition of catechins in *Camellia sinensis* and *Ilex latifolia* (mg g$^{-1}$)

| Species | GC | EGC | C | EC | EGCG | GCG | ECG | CG | Total |
|---|---|---|---|---|---|---|---|---|---|
| *C. sinensis* | 28.2 | 6.10 | 5.79 | 2.96 | 29.8 | 17.0 | 18.4 | 4.66 | 112 |
| *I. latifolia* | 0 | 0 | 7.89 | 5.62 | 0 | 0 | 1.87 | 1.53 | 16.9 |

Table 4
Flavonoid composition of *Ilex latifolia* and *Camellis sinensis* (mg kg$^{-1}$)

| Plant species | Rutin | Mericetin | Quercetin | Total |
|---|---|---|---|---|
| *I. latifolia* | 4050 | 675 | 145 | 4780 |
| *C. sinensis* | 101 | 404 | 74.5 | 580 |

### 2.6. Determination of polyphenols

Five grams of ground sample were extracted with 500 ml boiling distilled water in a boiling water bath for 10 min and then filtered through a "Double-ring" No. 102 filter paper. Polyphenol concentrations of the filtrates were determined by the spectrophotometric method described by Zhong (1989).

## 3. Results and discussion

### 3.1. Amino acids

Sixteen amino acids were found in *C. sinensis*, but fifteen in *I. latifolia* (Table 1). Theanine, which is a special amino acid in *Camellia* (Wu, 1980), was not found in *I. latifolia*. Amino acid contents were quite different between the two plant species. The total amino acids content was 31058 mg kg$^{-1}$ in *C. sinensis*, which was 15.8 times that of *I. latifolia*. Histidine, aspartic acid and glutamic acid were the major amino acids in *I. latifolia*, which accounted for 81.9% of its total amino acids content. Theanine and glutamic acid were the major amino acids in *C. sinensis*, which accounted for 54.4% of total amino acid content. Contents of phenylalanine, tyrosine, tryptophan, arginine, lysine and glycine were very low in *I. latifolia*.

### 3.2. Ascorbic acid, caffeine and polyphenols

Ascorbic acid was found in *I. latifolia*, but its content was only 0.46 mg g$^{-1}$ which was 22.1% of that in *C. sinensis*. Polyphenols content in *I. latifolia* was 90.1 mg g$^{-1}$, which was less than 50 percent of that in *C. sinensis* (Table 2).

HPLC profile of *I. latifolia* at 280 nm, differed obviously from that of *C. sinensis* (Fig. 1 A, C). There were only six major peaks in leaf extract of *I. latifolia* (Fig. 1C). Four evident peaks were resolved during retention times between 7 and 14 min in *C. sinensis* but not in *I. latifolia*. Peaks 5 and 6, the largest two peaks detected at 50.42 min and 53.69 min in *I. latifolia*, respectively, were very low in *C. sinensis* (Fig. 1).

It is well known that caffeine and catechins, a group of predominant polyphenol compounds in *C. sinensis*, are characteristic compounds in leaf of *C. sinensis*. Caffeine and eight tested catechins were detected in *C. sinensis* (Fig. 1; Table 3). Total content of the eight tested catechins accounted for 59.9% of polyphenols in *C. sinensis* (Tables 2 and 3). L-epigallocatechin gallate (EGCG) and L-gallocatechin (GC) were major catechins in *C. sinensis*, which accounted for 51.8% of total content of the eight tested catechins and 31% of polyphenols (Tables 2 and 3). L-gallocatechin gallate (GCG) and L-epicatechin gallate (ECG) were 17.0 mg g$^{-1}$ and 18.4 mg g$^{-1}$, respectively. L-epicatechin (EC), L-catechin (C), L-catechin gallate (CG) and L-epigallocatechin (EGC) were below 7 mg g$^{-1}$ (Table 3).

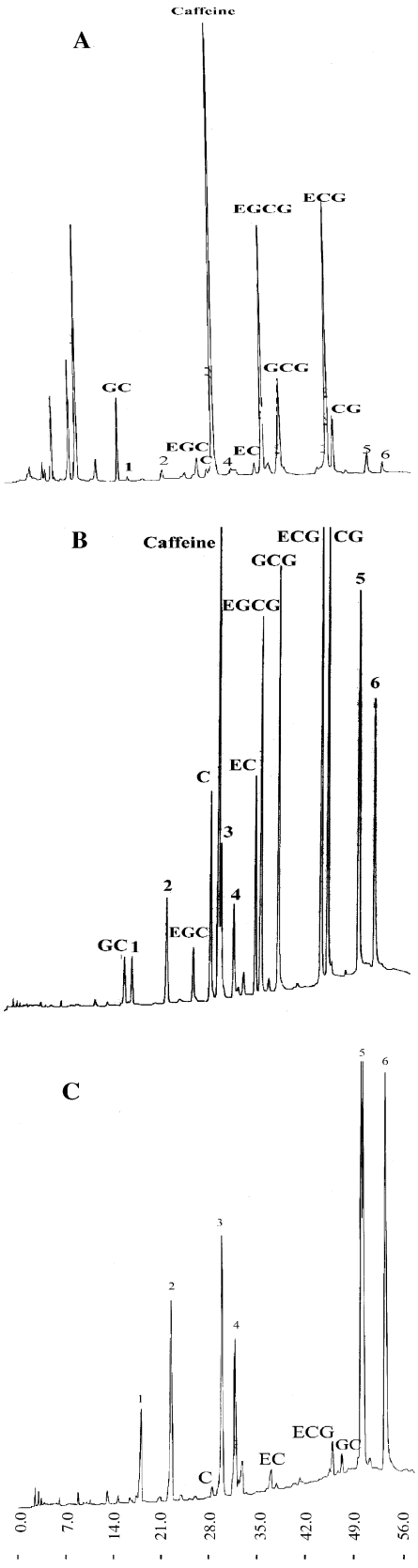


Fig. 1. Comparison of catechin compositions of *Ilex latifolia* and *Camellia sinensis*. (A) Extract of *Camellia sinensis* (10 μl); (B) Mixture of 5 μl extract of *Ilex latifolia* and 5 μl reference compounds; (C) Extract of *Ilex latifolia* (10 μl).

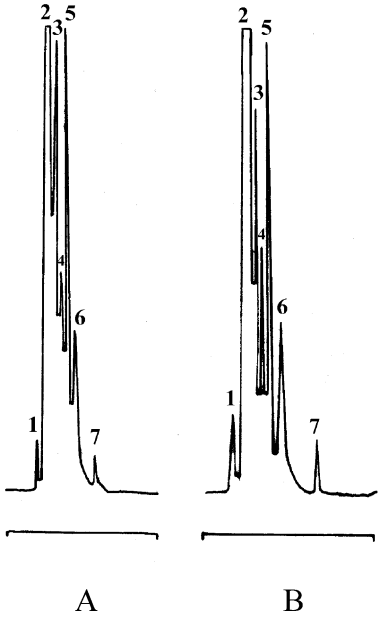


Fig. 2. HPLC diagram of flavonoids of *Camellia sinensis* (A) and *Ilex latifolia* (B).

In order to reveal whether leaf of *I. latifolia* contained the catechins and caffeine or not, a mixture of 5 μl leaf extract of *I. latifolia* and 5 μl reference compounds of the eight catechins and caffeine were injected into HPLC by the internal standard method (Liang & Liu, 1994). It was shown that *I. latifolia* had a low concentration of C, EC, CG or ECG, but no EGCG, GC, GCG or EGC (Fig. 1B; Table 3).

### 3.3. Flavonoid compounds

Flavonoid compounds have been shown to make important contributions to the quality of tea (*C. sinensis*; Sakamoto, 1967). Seven peaks were resolved by HPLC at 254 nm in the extracts of the two tested samples (Fig. 2). Peaks 4, 6 and 7 were identified as rutin, myricetin and quercetin using reference compounds. Apiolin, hesperetin and morin were also used as reference compounds in this test but they were not detected in the two samples. Peaks 2, 3 and 5 may be important flavonoid compounds in the *I. latifolia* and *C. sinensis* but, in the absence of suitable reference compounds, they remained unconfirmed. Contents of the three identified flavonoids differed markedly between *I. latifolia* and *C. sinensis*. Content of rutin was highest in *I. latifolia* but mericetin was highest in *C. sinensis* (Table 4). The total concentration of the three identified flavonoids in *I. latifolia* was 478 mg $kg^{-1}$, seven times greater than that in *C. sinensis*.

In conclusion, the chemical composition of *I. latifolia* leaf was greatly different from *C. sinensis* leaf. *I. latifolia* had more flavonoids, less amino acids and catechins than *C. sinensis* but no caffeine, EGCG, GC, GCG or

EGC, the four important catechins found in tea leaf. The higher level of flavonoids may be related to the healthy function and beverage value of *I. latifolia* leaf.

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