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Comparative Quantification of 14 Bioactive Compounds in Camellia ptilophylla and Camellia sinensis

Sihui Ying
South China Agricultural University
Qiuyan Huang
South China Agricultural University
Sen Lu
South China Agricultural University
Xiong Gao
Guangdong Academy of Sciences
Zhongzheng Chen
South China Agricultural University
Yuanyuan Zhang
South China Agricultural University
Xiaoshan Liu
South China Agricultural University
Bin Li
South China Agricultural University
Xiaorong Lin (xiaoronglin@scau.edu.cn)
South China Agricultural University

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Abstract

Camellia ptilophylla is a rare tea species discovered in southern China which naturally containing low caffeine but high theobromine, high *trans*-catechin, and two special polyphenols, gallocatechin-3,5-digallate and 1,2,4,6-tetragalloyl glucose. But the commonly-used methods for the quantification of tea bioactive compounds do not cover these two special polyphenols, limiting the systematical phytochemical analysis of *Camellia ptilophylla*. Herein, we explored a high-performance liquid chromatography-based method to quantify 14 bioactive compounds including three methylxanthines, eight catechin monomers, gallic acid, gallocatechin-3,5-digallate, and 1,2,4,6-tetragalloyl glucose, and applied it on *Camellia ptilophylla* and six typical Chinese teas from *Camellia sinensis*. The present method facilitated the effective separation of 14 tea bioactive compounds within 35 min with good linearity (R^2 ≥0.9999), excellent sensitivity and reproducibility with a limit of detection of 1.2272-19.7611 ng/mL and a relative standard deviation lower than 4.1%, and satisfactory recoveries of the spiked samples of 95.57%-106.93% with the relative standard deviation lower than 5%. Basing on this method, the characteristics of high theobromine (3.4-4.8%), low caffeine (0.01-0.2%), high gallocatechin gallate (6.3-8.1%) and catechin (C, 2.2-3.4%), low epigallocatechin gallate (2.2-3.0%) and epicatechin (0.3-0.6%), and containing gallocatechin-3,5-digallate (0.6-0.9%) and 1,2,4,6-tetragalloyl glucose (0.5-1.1%) of *Camellia ptilophylla* were confirmed. This unique profile of bioactive compounds was not found in six typical Chinese teas from *Camellia sinensis*. In conclusion, theobromine, gallocatechin gallate, catechin, gallocatechin-3,5-digallate, and 1,2,4,6-tetragalloyl glucose with steadily high content were recognized as the characteristic bioactive components of *Camellia ptilophylla*.

Introduction

Tea, which originated from China, has become one of the most popular non-alcohol beverages in the world. This worldwide prevalence of tea derived from the wide consensus of consumers about its health-beneficial effects. Tea polyphenols and methylxanthines have been recognized as the dominant chemical basis of the health functions of tea including anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-obesity effects, etc(Tang et al. 2019). Thus, the detection of tea polyphenols and methylxanthines provides key indications of the bioactivities of teas. Though tea polyphenols and methylxanthines comprise various monomers, only caffeine (Caf) and four *cis*-type catechins including (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) (Fig. 1) are generally determined as they dominate the content of methylxanthines (90%-95%) and tea polyphenols (50%-60%) respectively in common tea varieties(Determination of substances characteristic of green and black tea - Part 2: Content of catechins in green tea - Method using high-performance liquid chromatography).

However, benefiting from the efforts of breeding, an increasing number of special tea varieties have been reported. Amongst, *Camellia ptilophylla* Chang (*C. ptilophylla*) discovered in 1981 in South China(Zhang H 1981) attracted the most attention in the recent decade. *C. ptilophylla* contained extremely low Caf (0-0.1%) but high theobromine (Tb, 3–7%) instead. In addition, *trans*-catechins including (+)-catechin (C),

(+)-gallocatechin (GC), (-)-catechin gallate (CG), and (-)-gallocatechin gallate (GCG) (Fig. 1) are found as the predominant catechins (80% of total polyphenols) in *C. ptilophylla*(Lin et al. 2014). Moreover, two novel phenolic compounds involving (-)-gallocatechin-3,5-di-O-gallate (GC-3,5-diGA), and 1,2,4,6-Tetra-O-galloyl-β-D-glucopyranose (1,2,4,6-GA-glc) are discovered in the extracts of *C. ptilophylla*(Kurihara et al. 2006). This peculiar chemical composition provides *C. ptilophylla* with dramatically higher anti-angiogenesis(Gao et al. 2020; Li et al. 2017), anti-oxidant(Kuang et al. 2020), anti-inflammatory(Gao et al. 2017), and hypolipemic(Kurihara et al. 2006) bioactivity but much lower central nervous exciting effect(Xu et al. 1990) compared to common tea varieties. Accordingly, accurate quantification of these dominant bioactive compounds in *C. ptilophylla* is required for the evaluation of its health effects.

Herein, we explored a high-performance-liquid-chromatography (HPLC)-based approach for the simultaneous detection of 11 tea polyphenols and 3 methylxanthines (Fig. 1). Additionally, method validation was further performed by the evaluation of the linearity, precision, repeatability, extraction recovery, the limit of detection (LOD), and the limit of quantification (LOQ). On this basis, the method was applied to the water exacts of tea leaves from *C. ptilophylla* and six typical Chinese teas including green tea, oolong tea, black tea, white tea, yellow tea, and dark tea from *C. sinensis* to compare the patterns of major bioactive compounds. The present study enables the feasibility of rapid quantification of 14 major bioactive compounds in various types of teas from *C. ptilophylla* and *C. sinensis* and benefits the discovery of novel special tea varieties.

Materials And Methods

Tea Samples and Chemicals

Green tea of *C. ptilophylla* processed by our group previously in different years (2014, 2015, 2016, 2018, 2019, 2020, and 2021) were sealed and stored at 4°C. Six typical Chinese teas, including Fuding Dabai white tea, Junshan Yinzhen yellow tea, Yunnan large-leaf green tea, Yunnan large-leaf black tea, Jinguanyin oolong tea, and Yunnan Pu-erh dark tea, were obtained from Beijing Yunkai Yaji Tea Culture Center (Beijing, China), Guangdong Huahai Sugar Development Co., Ltd (Xuwen, Guangdong, China), Huaiji Gaoshanqing Agricultural Products Co., Ltd (Zhaoqing, Guangdong, China) and Guangdong Tea Import & Export Co., Ltd (Guangzhou, Guangdong, China), respectively.

GA (≥ 98%), Tb (≥ 98%), Caf (≥ 98%), and Tp (≥ 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). EC (≥ 98%), EGC (≥ 98%), EGCG (≥ 98%), ECG (≥ 98%), CG (≥ 98%), GCG (≥ 98%), GC (≥ 98%), and C (≥ 98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). GC-3,5-diGA and 1,2,4,6-GA-glc from *C. ptilophylla* were purified in our laboratory by preparative HPLC. HPLC grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (HPLC grade) and Folin-Ciocalteu Reagent (1 mol/L) were obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Ultrapure water was prepared via a Millipore Milli-Q purification system (Millipore Corp, Bedford, MA).

Preparation of Standard Solutions and Tea Samples

GA, Tb, Tp, Caf, C, EC, GCG, EGCG, CG, ECG, 1,2,4,6-GA-glc, GC-3,5-diGA, GC, and EGC were dissolved in 20% (w/w) acetonitrile and diluted to obtain a series of working solutions. Tea leaves (0.3 g), ground and sieved ranging from 20 to 30 mesh, were extracted with 45 mL of ultrapure water in a boiling water bath for 45 min. Subsequently, cooled tea extracts were fixed to 50 mL and diluted 1-fold with water followed by the filtration with a 0.22 μm PES filter (Anpel, Shanghai, China).

HPLC Determination of 14 Bioactive Compounds

The HPLC analysis of tea polyphenols and methylxanthines was performed on a Shimadzu LC 2030C 3D plus workstation, including a Shimadzu Prominence-i LC-2030C 3D chromatography, a PDA detector, a column oven, and an autosampler (Shimadzu, Kyoto, Japan). Samples (10 μL) were eluted on an Agilent Poroshell 120 Bonus-RP (4.6 mm×50 mm, 2.7μm) column with trifluoroacetic acid aqueous solution (0.05%, solvent A) and acetonitrile (solvent B) at 30°C with a flow rate of 0.8 mL/min. The gradient elution was performed as follows: 100%A from 0 to 8 min, 100%A decreased to 91%A from 8 to 17 min, 91%A decreased to 82%A from 17 to 25 min, 82%A decreased to 76% A from 25 to 30 min, and 76% A decreased to 72% from 30 to 35 min. The absorbance at 280 nm was recorded and calculated with LabSolutions 5.8 (Shimadzu).

The linearity, precision, repeatability, extraction recovery, LOD, and LOQ were evaluated for method validation. Relative standard deviations (RSDs) of the peak areas from five injections of the mix solutions of 14 standards were calculated to access the precision. RSDs of the 14 compounds in five replicate tea extracts from *C. ptilophylla* were used for the repeatability evaluation. To determine the extraction recovery, tea extracts of *C. ptilophylla* mixed 1:1 with standard mixtures as the spiked group, tea extracts of *C. ptilophylla* mixed 1:1 with 20% (w/w) acetonitrile as the background group, and standard mixtures mixed with 2% (w/w) acetic acid in equal volume as the standard group were tested and compared. LOD and LOQ were obtained from the concentration of standard solutions diluted with 20% (w/w) acetonitrile when the signal-to-noise ratio (S/N) reached 3 and 10, respectively.

Determination of the Content of Total Polyphenol and Water Extract

The content of total polyphenols of green tea from *C. ptilophylla* and six typical teas from *C. sinensis* were determined via the colorimetric method using Folin-Ciocalteau reagent(Determination of substances characteristic of green and black tea — Part 1: Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent 2005). The water extract content was calculated based on the weight of tea hot-water-insoluble residues according to ISO 9768: 1994(Tea — Determination of water extract 1994) with a slight modification. Tea leaves were extracted using a boiling water bath and the residues were dried at 120 ± 2°C.

Statistical Analysis

All data from two individual measurements with three replicates were represented as mean values ± standard deviation (SD). The significance of differences among various samples was evaluated by

Duncan's multiple range test using SPSS 20.0.

Results

Simultaneous HPLC Determination of 14 Tea Compounds

Despite the remarkable difference in the hydrophilicity and hydrophobicity among 14 tea bioactive compounds, they were perfectly eluted within 35 min on the reversed-phase column by 0.05% trifluoroacetic acid aqueous solution and acetonitrile in the present method (Fig. 1A). When the method was applied to the water extracts from *C. ptilophylla* and *C. sinensis*, all of these bioactive compounds were also effectively separated (Fig. 1B and Fig. 1C). It indicated that the detection of these 14 targeted compounds was not affected by the complexity of the tea matrix. Additionally, the HPLC chromatography of *C. ptilophylla* confirmed its high content of Tb and *trans*-type catechins but low content of Caf and *cis*-catechins. Furthermore, the presence of two novel compounds, GC-3,5- diGA and 1,2,4,6-GA -glc, in *C. ptilophylla* but not *C. sinensis* were also verified. This result preliminarily suggested the feasibility of this HPLC-based method for the rapid detection of 14 major bioactive compounds in *C. ptilophylla* and *C. sinensis*.

To further validate the method, linearity, precision, repeatability, extraction recovery, LOD and LOQ were calculated (Fig. 2 and Table 1). Excellent linearity was observed between the concentration and the peak area of 14 compounds with the values of R-square higher than 0.999 (Fig. 3). A satisfactory precision was additionally suggested by the RSDs of all targeted compounds ranging from 0.12–1.08% for five consecutive tests (Table 1). Furthermore, the sound repeatability of this method was confirmed by the low RSDs of each component from tea samples extracted five times (Table 1). Moreover, the spiked recoveries of the 14 compounds ranging from 95.57–106.93% with the RSD values of 0.21%-4.88% also satisfied the requirement of the accurate quantification of 14 bioactive compounds in tea extracts (Table 1). The high sensitivity of this method was further revealed by the LODs of these compounds ranging from 1.2272 ng/mL to 19.7611 ng/mL and their LOQs of 17.2410-76.2649 ng/mL (Table 1). These results suggested the effectiveness, efficiency, accuracy, and reliability of this method in the quantitative analysis of 14 major tea polyphenols and methylxanthines.

Table 1
Method validation

Compounds	Precision RSD (%)	Repeatability RSD (%)	Recovery (%)	Recovery RSD (%)	LOD (ng/mL)	LOQ (ng/mL)
GA	0.17	2.98	103.07	1.06	4.16	17.24
Tb	0.17	2.07	102.13	1.18	8.97	21.16
Tp	0.13	3.07	106.93	0.21	1.23	10.31
GC	0.57	2.78	103.52	4.88	19.76	66.20
Caf	0.16	1.65	99.59	1.30	2.86	16.47
EGC	0.14	3.90	101.53	3.80	10.58	52.92
C	0.12	2.21	96.76	0.28	14.90	76.26
EC	0.32	2.30	96.12	1.19	17.98	44.37
EGCG	0.34	2.81	95.57	1.55	6.60	25.79
GCG	0.22	4.06	102.03	0.51	7.14	21.42
ECG	0.29	1.01	97.23	0.51	10.47	31.41
1,2,4,6-GA-glc	1.08	3.60	97.26	1.18	7.90	27.64
CG	0.30	3.15	98.86	1.92	6.37	28.54
GC-3,5-diGA	1.07	2.52	95.81	3.86	11.85	35.54

Relative Correction Factors of Two Novel Compounds

As the reference standards of two novel phenolic compounds, GC-3,5-diGA and 1,2,4,6-GA-glc, were not commercially accessible, a feasible approach to access the content of these two monomers in tea samples was explored based on their relative correction factors to the other 12 compounds (Table 2). As suggested by the European Pharmacopoeia (EP10.0), the targeted compounds can be quantified using the commercial standards of the reference chemicals if their relative correction factors range from 0.8 to 1.2. Accordingly, Caf and Tp as well as ECG and CG were recommended to be the reference compounds for 1,2,4,6-GA-glc and GC-3,5-diGA, respectively (Table 2).

Table 2
Relative correction factors of two novel bioactive compounds discovered in *C. ptilophylla* to other 12 commercial reference compounds

Reference compounds	Relative correction factor	
	1,2,4,6-GA-glc	GC-3,5-diGA
GA	1.36	1.74
Tb	1.32	1.70
Caf	1.17	1.50
Tp	1.10	1.42
CG	0.74	0.95
ECG	0.67	0.86
GCG	0.59	0.76
EGCG	0.54	0.69
EC	0.28	0.36
C	0.27	0.34
GC	0.07	0.09
EGC	0.07	0.09

Composition of Bioactive Compounds in C. ptilophylla **and** C. sinensis

To profile the composition of bioactive compounds in *C. ptilophylla*, 14 compounds in green tea from *C. ptilophylla* and six typical Chinese teas from *C. sinensis* including green tea, oolong tea, black tea, white tea, yellow tea, and dark tea were detected and their percentage in the total water extracts were compared in Fig. 4. The 14 bioactive compounds were found to account for about 40% of the total water extract of green tea from *C. ptilophylla*. Amongst, tea polyphenols (33.1%), especially eight catechin monomers (30.6%), were recognized as the dominant bioactive compounds. In detail, the percentages of gallated catechins (19.8%), 2,3-*trans* structure (20.5%), and the pyrogallol-type catechins (23.7%) were higher than ungallated (10.8%), 2,3-*cis* (10.1%), and the catechol-type (7.0%). And GCG (13.5%) was found the predominant bioactive compound. In addition, the percentage of all methylxanthines in the water extracts of *C. ptilophylla* reached 7.2%, of which Tb showed the highest percentage of 7.0%.

In the water extracts of all six typical Chinese teas, two special polyphenols, 1,2,4,6-GA-glc and GC-3,5-diGA, were not detected. Additionally, the bioactive compounds in green tea accounted for the highest percentage of the total water extract (38.5%), followed by oolong tea (29.3%), yellow tea (21.0%) and white tea (20.6%), black tea (8.8%) and dark tea (7.3%) the lowest. This difference mainly came from the different percentages of catechins in water extracts as shown in the following order: green tea (32.3%) >

oolong tea (24.7%) > yellow tea (12.9%) and white tea (12.6%) > black tea (2.3%) > dark tea (0.8%). Furthermore, the percentages of methylxanthines in water extracts of six teas were found from 4.5–7.2%. Compared with green tea from *C. ptilophylla*, the percentage of catechins in the water extract of green tea from *C. sinensis* was higher. Among these catechins, the percentages of gallated catechins (19.7%), the 2,3-*cis* structure (21.7%), and the pyrogallol type (18.1%) were higher than those of ungallated (12.6%), 2, 3-*trans* (10.6%), and catechol (13.3%) type, with EGCG accounting for the highest (8.0%). However, the percentage of methylxanthines in *C. sinensis* (5.9%) was slightly lower than that of *C. ptilophylla*, with Caf accounting for the highest percentage (5.8%).

This result proved the feasibility of this method in the detection of bioactive compounds in *C. ptilophylla* and *C. sinensis* without interference from different tea trees and processing methods. It also confirmed the unique composition of bioactive compounds of *C. ptilophylla*. Amongst these compounds, 1,2,4,6-GA-glc and GC-3,5-diGA have demonstrated the unique bioactive compounds of *C. ptilophylla*.

To further confirm the unique composition of *C. ptilophylla*, the content of 14 bioactive compounds in seven green teas of *C. ptilophylla* prepared in different years (2014–2021) was determined (Fig. 5). Most of the polyphenol monomers including GCG, EGCG, GC-3,5-diGA, C, EC, EGC, GA, 1,2,4,6-GA-glc, and GC in the green teas of *C. ptilophylla* showed differences less than 50% in their content. Amongst, the content of GCG, ranging from 6.3–8.2%, showed only a slight difference of less than 15%. But the content of ECG and CG in different green teas from *C. ptilophylla* fluctuated the most (reached 85%), which was mostly induced by the samples made in 2021. Among the three typical methylxanthines, the fluctuation range of Tb content is less than 18%, while the fluctuation range of Caf and Tp is much larger. Regardless, the Caf content of all green teas from *C. ptilophylla* was less than 0.2%, much lower than the breeding standard for the low-caffeine contained tea variety of 1%. These results indicated that the content of GCG and Tb, the two characteristic components of *C. ptilophylla*, are relatively stable. Though the Caf content in different green teas from *C. ptilophylla* fluctuated significantly, its phytochemical specificity of high Tb but low Caf did not change.

Discussion

Tb is a typical methylxanthine in cocoa with beneficial effects on cardiovascular, respiratory, renal, etc(Smit 2011). Generally, the content in tea trees is less than 0.1%. Ma and Zhang (1984) first reported Tb as the predominant methylxanthine of *C. ptilophylla* with a content of 4.4% (dry weight) (Ma Y and Zhang R 1984). Subsequently, they compared the composition of methylxanthines of *C. ptilophylla* with seven green tea, oolong tea, and black tea from *C. sinensis* using the HPLC method, and confirmed that only *C. ptilophylla* contained high Tb (3.02 ~ 4.68%), which was 11.7 ~ 15.1 times higher than other teas from *C. sinenesis*(Ma Y and Chen Y 1996). Gao et al. (2004) found that both cultivated and wild *C. ptilophylla* contained high Tb, but the Tb content in the cultivated tea trees was slightly lower than the latter He et al. (2011) indicated that the characteristic of high-Tb of *C. ptilophylla* was not affected by the planting seasons and the processing methods, but the Tb content of *C. ptilophylla* plucked in summer (6.06–6.26%) was higher than those plucked in spring (5.25–5.81%) and autumn (4.65 ~ 5.31%). The

present study confirmed that the Tb content of different green teas from *C. ptilophylla* was 3.4–4.8% with a difference of less than 20%. These results demonstrated that Tb was a characteristic component of *C. ptilophylla*, even though the content of Tb might slightly vary due to harvest seasons and cultivation methods.

The particular composition of catechins in *C. ptilophylla* was not found in the early days due to the failure of Ultra Violet-Visible Spectroscopy to distinguish the *cis*- and *trans* catechin monomers(Li B et al. 2001; Gao K et al. 2004). In 2006, Kurihara et al. (2006) first indicated that the GCG content in the water extract of *C. ptilophylla* (199 mg/g) was 53 times higher than that of *C. sinensis*, but the contents of EGCG and EGC in *C. ptilophylla* extract were only a half of that of *C. sinensis*. Yang et al. (2007) further proved the high content of *trans*-type catechins in *C. ptilophylla*. They found that the contents of GCG (9.88%) and C (2.23%) in *C. ptilophylla* were 7- and 4-time higher than those of *C. sinensis*, respectively. The GC content of *C. ptilophylla* (1.17%) is comparable to that of *C. sinensis*, while the contents of EGCG, EGC, EC, and ECG were much lower than those of the latter. In addition, Peng et al. (2008,2011) indicated that the contents of GCG (7.4–7.6%), C (2.6–3.4%), and GC (1.5–2.7%) in *C. ptilophylla* were significantly higher than those of *C. sinensis*, but its CG content (006-0.1%) was similar to that of the latter. In the study of Li et al. (2012), they found that the contents of GCG, C, GC, and CG in *C. ptilophylla* were 8.11%, 3.26%, 1.67%, and 0.35%, respectively. In the present investigation, GCG and C were found the first two dominant catechin monomers in *C. ptilophylla* as their contents were as high as 6.3–8.1% and 2.5–3.5% with a slight difference less than 20%. But the contents of GC (0.5–1.2%) and CG (0.1–0.3%) in different green teas of *C. ptilophylla* were much lower with a great fluctuation as high as 80%. It could be concluded according to the above studies that GCG and C with a steadily high content were the characteristic catechins of *C. ptilophylla*.

Fukui et al. (2005)first found the two special tea polyphenols, GC-3,5-diGA and 1,2,4,6-GA-glc, in the extract of *C. ptilophylla* with a content of 0.3% and 0.5%, respectively(Kurihara et al. 2006). On this basis, our previous study further confirmed the stronger antioxidant potentials of GC-3,5-diGA and 1,2,4,6-GA-glc purified from the ethanol/ethyl acetate extract of *C. ptilophylla* compared to GCG via the *in vitro* cell lines, indicating that these two special polyphenols were key bioactive compounds of *C. ptilophylla*(Kuang et al. 2020). Thus, we proposed an HPLC-based quantitative method for the above major bioactive compounds of *C. ptilophylla*, confirming that GC-3,5-diGA and 1,2 ,4,6-GA-glc were only found in *C. ptilophylla* with their contents of 0.6–0.9% and 0.5–1.1% but not detected in the six typical Chinese teas from *C. sinensis*. These results indicated GC-3,5-diGA and 1,2 ,4,6-GA-glc were also the characteristic phytochemical markers of *C. ptilophylla*.

Conclusions

In conclusion, an HPLC strategy was developed in this study to quantify 14 bioactive compounds in *C. ptilophylla* and *C. sinensis* within 35 min. Based on this method, we verified that the particularity of high Tb content, high *trans*-catechins (GCG and C) content, and two special polyphenols of *C. ptilophylla* was not observed in the six typical Chinese teas of *C. sienensis*.

Declarations

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Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interests Sihui Ying declares that she has no conflict of interest. Qiuyan Huang declares that she has no conflict of interest. Sen Lu declares that he has no conflict of interest. Xiong Gao declares that he has no conflict of interest. Zhongzheng Chen declares that he has no conflict of interest. Yuanyuan Zhang declares that she has no conflict of interest. Xiaoshan Liu declares that she has no conflict of interest. Bin Li declares that she has no conflict of interest. Xiaorong Lin declares that she has no conflict of interest.

Ethics Approval and Consent to Participate This article does not contain any studies with human participants or animals performed by any of the authors.

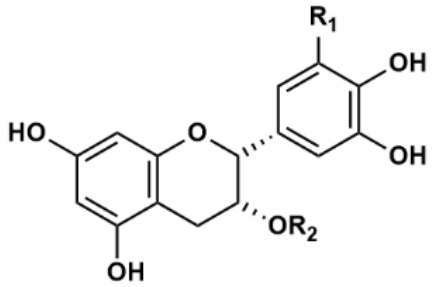
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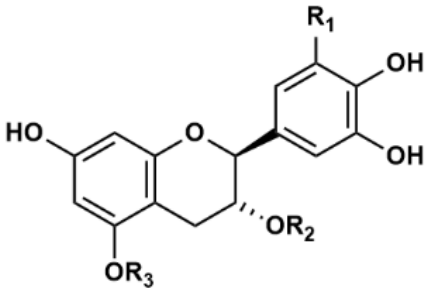
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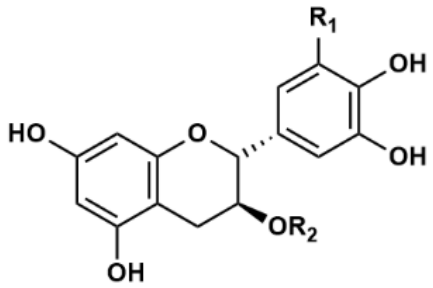
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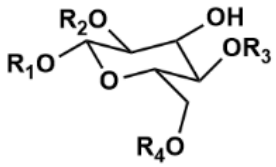


R_1=R_2=H: (-)-Epicatechin (EC)
R_1=OH, R_2=H: (-)-Epigallocatechin (EGC)
R_1=H, R_2=D: (-)-Epicatechin gallate (ECG)
R_1=OH, R_2=D: (-)-Epigallocatechin gallate (EGCG)



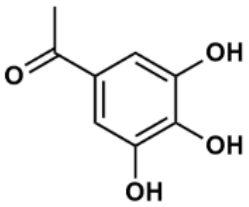
R_1=R_2=H: (-)-Catechin
R_1=OH, R_2=H: (-)-Gallocatechin (GC)
R_1=H, R_2=D, R_3=H: (-)-Catechin gallate (CG)
R_1=OH, R_2=D, R_3=H: (-)-Gallocatechin gallate (GCG)
R_1=OH, R_2=R_3=D: (-)-Gallocatechin-3,5-di-*O*-gallate (GC-3,5-diGA)



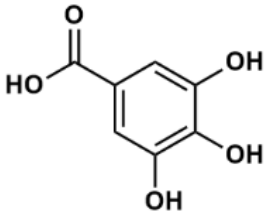


R_1=R_2=R_3=R_4=D: 1,2,4,6-Tetra-*O*-galloyl-β-D-glucopyranose (1,2,4,6-GA-glc)

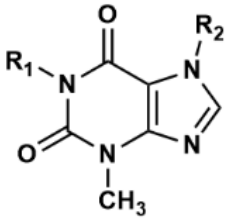
R_1=OH, R_2=H: (+)-Gallocatechin (GC)
R_1=R_2=H: (+)-Catechin (C)



D: Galloyl group



Gallic acid (GA)



R_1=H, R_2=CH_3: Theobromine (Tb)
R_1=CH_3, R_2=H: Theophylline (Tp)
R_1=CH_3, R_2=CH_3: Caffeine (Caf)

Figure 1

Chemical structure of 14 tea polyphenols and methylxanthines

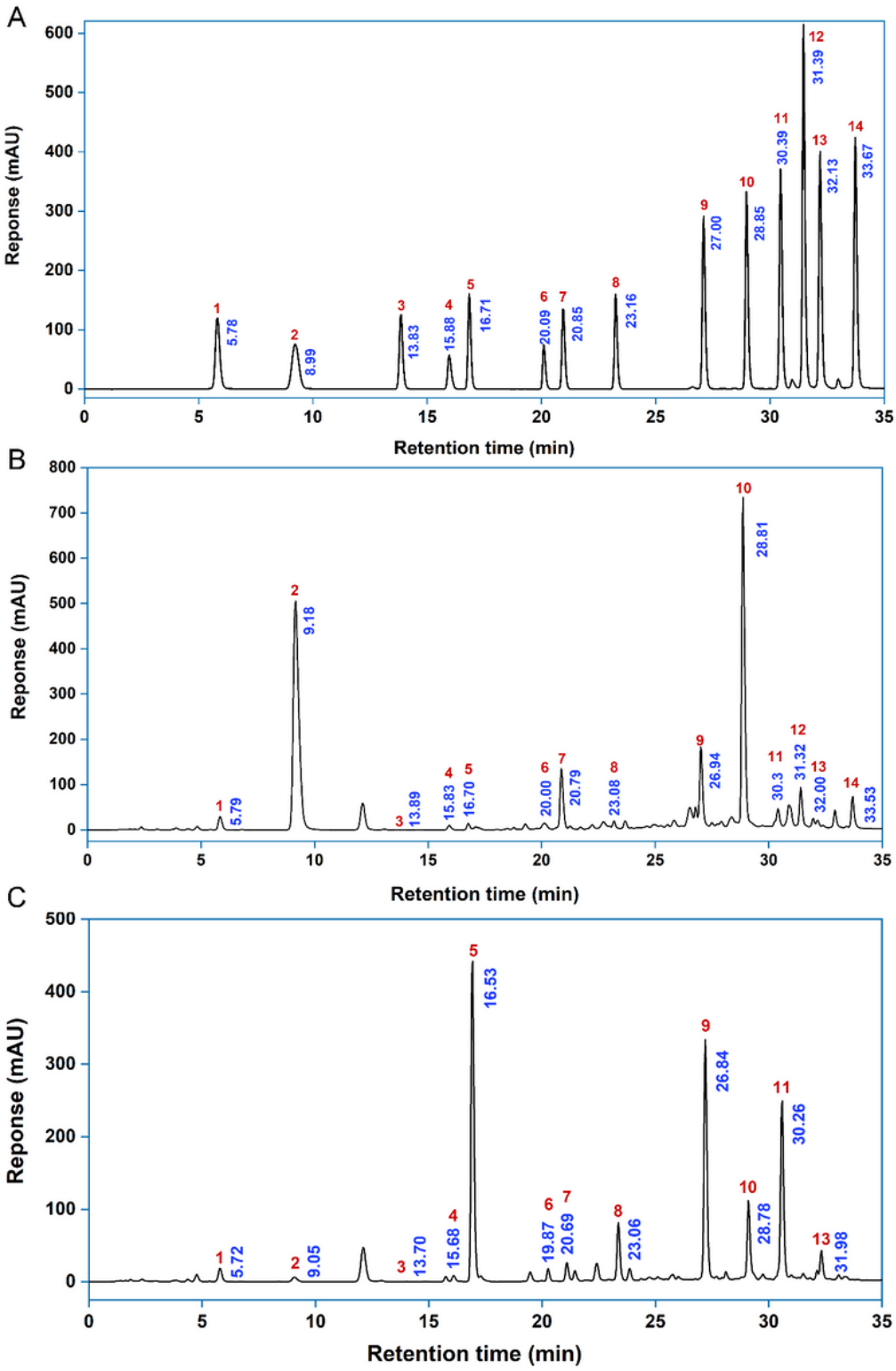


Figure 2

HPLC chromatograms of standards (A), *C. ptilophylla* (B), and *C. sinensis*(C)

Peak 1-14 denoted orderly as GA, Tb, Tp, GC, Caf, EGC, C, EC, EGCG, GCG, ECG, 1,2,4,6-GA-glc, CG, and GC-3,5-diGA.

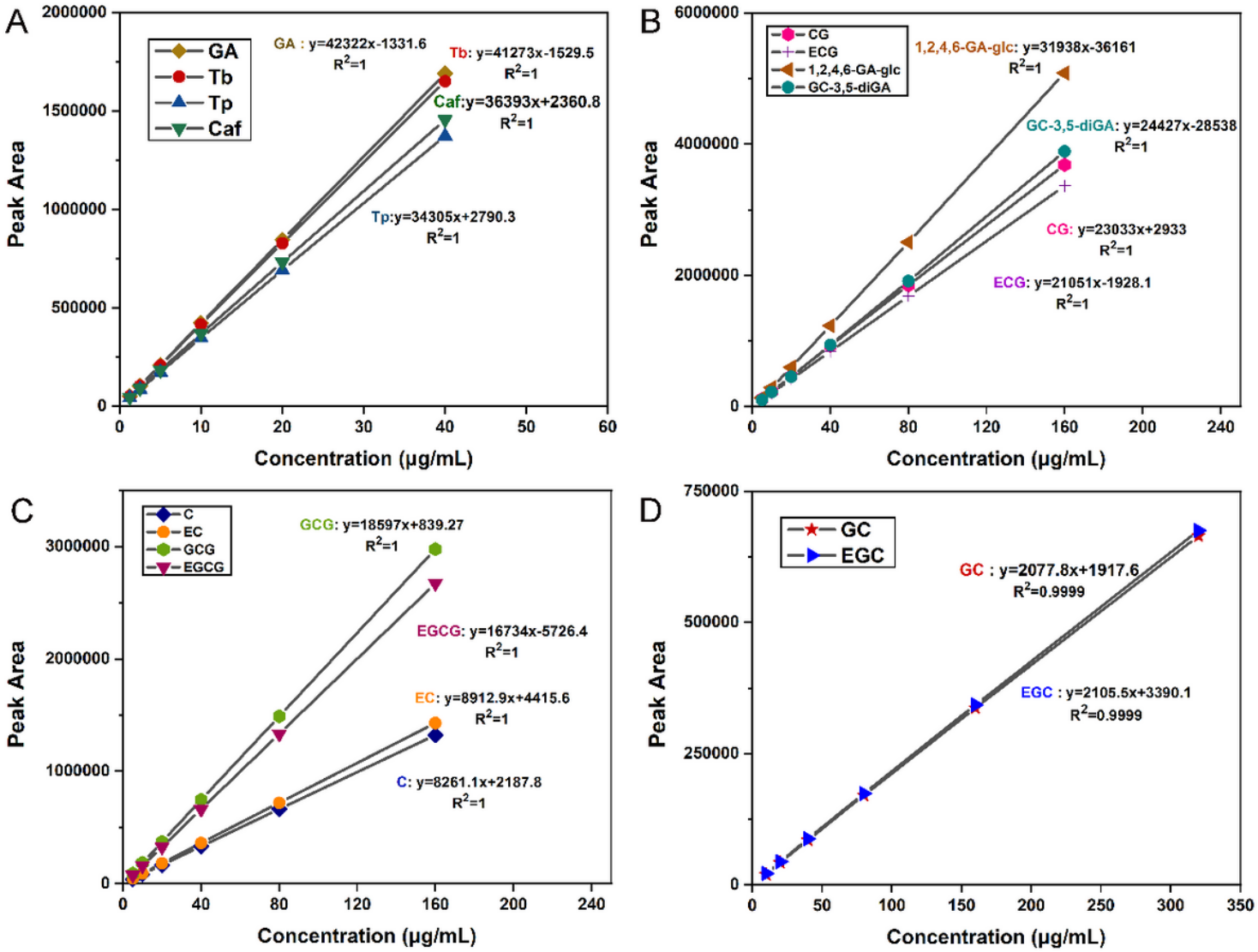


Figure 3

Standard curves of 14 bioactive compounds

(A) for GA, Tb, Tp, and Caf ranges from 1.25 μg/mL to 40 μg/mL; (B) for CG, ECG, 1,2,4,6-GA-glc, and GC-3,5-diGA of 5-160 μg/mL; (C) for C, EC, GCG, EGCG of 5-160 μg/mL; (D) for GC and EGC from 10 μg/mL to 320 μg/mL.

Figure 4

Percentage of 14 bioactive compounds in total water extracts of green tea from *C. ptilophylla* and six typical teas from *C. sineneis*

Figure 5

Differences in the content of 14 bioactive compounds in different green teas from *C. ptilophylla*