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Chemical composition, sensory properties and bioactivities of *Castanopsis lamontii* buds and mature leaves

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ABSTRACT

Castanopsis lamontii is used as functional herbal tea in southwest China. Usually, only buds rather than mature leaves are applied. To figure out whether mature leaves were suitable for producing herbal tea, chemical composition, sensory properties and bioactivities of *Castanopsis lamontii* bud infusion (CLB) and mature leaf infusion (CLM) were investigated. According to the results, CLB and CLM had similar non-volatile composition, but in different proportion. Meanwhile, CLB contained more types of volatiles than CLM, leading to distinguishable volatile profiles between them. Sensory assessment showed that CLB had sweet aftertaste and floral aroma. CLM tasted astringent and smelled grassy. Bioactivity evaluation indicated that CLB exhibited higher activities in scavenging free radicals and suppressing lipopolysaccharide-induced inflammation. Taken together, CLB had better overall acceptability in sensory quality and higher bioactivity, implying that *Castanopsis lamontii* buds were more suitable for producing herbal tea.

1. Introduction

Castanopsis lamontii is the raw material of a herbal tea called "*Shaji* (in Chinese)", which has been used to refresh breath and prevent oral inflammation for ages in southwest China. *Castanopsis lamontii* belongs to the *Fagaceae* family. Many members in this family are rich in functional components (e.g., polyphenols, triterpenoids and flavones), and their extracts exhibit multiple biological activities (e.g., anti-inflammatory and antioxidant) (Gao et al., 2019; Khan, Kihara, & Omoloso, 2001; Yadav & Tangpu, 2007). Usually, buds rather than mature leaves of *Castanopsis lamontii* were collected and brewed for use. The *Castanopsis lamontii* bud infusion (CLB) has bright color, delightful smell and pleasant sweet aftertaste. Our former study demonstrated that polyphenols, soluble sugars and saponins were the predominant components in the *Castanopsis lamontii* bud water extract (Gao et al., 2019). Our study also provided experimental evidence of the bioactivities of *Castanopsis lamontii* bud water extract, proving its effectiveness for inhibiting pathogens, inflammation and oxidative damage, and (–)-epicatechin and procyanidin B2, were proved to contribute to these bioactivities (Gao et al., 2019).

However, little information about the chemical profiles, sensory properties and bioactivities of the *Castanopsis lamontii* mature leaf infusion (CLM) has been reported. Whether it's reasonable to only use the buds but not mature leaves of *Castanopsis lamontii* to produce herbal tea remains unclear. To figure it out, the chemical composition, sensory quality and bioactivities of these two infusions were investigated and compared. General chemical analysis, high-performance liquid chromatography (HPLC) and UPLC-QE-Orbitrap-MS analysis were conducted to determine the non-volatile chemical composition. Gas chromatography-mass spectrometry (GC–MS) was used to analyze volatile components. Taste and odor sensory tests were carried out to assess the sensory quality. The color of infusion was determined by a spectrophotometer. Superoxide anion scavenging activity, hydroxyl radical scavenging activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were measured to evaluate antioxidant activities. The inhibitory activities of lipopolysaccharide-induced nitric oxide (NO), prostaglandin E2 (PGE2), and tumor necrosis factor α (TNF-α) secretion were used to assess the anti-inflammatory activities. The results will increase the understanding of differences between *Castanopsis lamontii* buds and mature leaves, and guide tea makers in selecting proper raw materials for producing high quality *Shaji* herbal tea.

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2. Materials and methods

2.1. Reagents

Standard epicatechin, procyanidin B2, catechin, quinic acid, malic acid, citric acid, succinic acid, gallocatechin, epigallocatechin, chrysoeriol, madecassic acid, asiatic acid, DPPH and lipopolysaccharides (LPS) were purchased from Sigma-Aldrich China-Mainland (Shanghai, China).

2.2. Preparation of infusions

The buds and mature leaves of *Castanopsis lamontii* were collected in the Dehong Area, China (24°17′12″ N, 98°23′6″ E). The buds and mature leaves were sorted, dried (110 °C for 30 min and then 80 °C for 60 min), powdered, and filtered (60-mesh), brewed with distilled water (1:100 w/v) at 80 °C for 20 min, cooled to room temperature, and centrifuged at 8000 × *g* for 10 min to obtain clear supernatant.

2.3. Determination of general non-volatile chemical composition

The concentrations of total polyphenols, soluble sugars, soluble polysaccharides, saponins, free amino acids, soluble proteins and flavones were determined based on the methods described previously (Gao et al., 2019).

Briefly, the total phenolic and free amino acid concentrations were measured according to the Chinese Standard of GB/T 8313-2008 and GB/T 8314-2013, respectively.

The soluble protein concentration was measured using a Bradford Protein Assay Kit purchased from Beyotime Biotechnology (Haimen, China).

The concentrations of soluble sugars and polysaccharides were measured using the anthrone-sulfuric acid method. For determining the concentration of soluble sugars, 1 mL sample solution was mixed with 4 mL anthrone-sulfuric acid (2 mg/mL) and incubated at 100 °C for 10 min. After cooling to room temperature, the absorbance was read at 620 nm. For determining the concentration of soluble polysaccharides, sample solution was mixed with 95% ethanol (v/v = 1:5), stored at 4 °C overnight, and centrifuged to spin down the polysaccharides. Dissolved the polysaccharides with appropriate amount of distilled water, and then analyzed it with the anthrone-sulfuric acid method.

The concentration of saponins was measured as the following procedure. A 200 μL sample solution was added to 0.5 mL 8% vanillin-alcohol solution and 3 mL 70% sulfuric acid and the reaction system was incubated at 60 °C for 10 min. After cooling to room temperature, the absorbance was read at 540 nm.

The concentration of flavones was measured with a modified Down method. 0.5 mL sample solution was mixed with 10 mL 1% aluminium trichloride ($AlCl_3$) and reacted at room temperature for 10 min. The absorbance was measured at 420 nm.

The concentrations of major polyphenols (i.e., epicatechin, procyanidin B2 and catechin) were measured using a previously established HPLC method (Hu et al., 2015).

UPLC-QE-Orbitrap-MS was used for untargeted analysis of the infusions. The separation was performed on a ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 100 mm, Waters, Milford, MA, U.S.A.) using a Dionex Ultimate 3000 RS system (Thermo Fisher). The gradient separation was carried out using 0.1% formic acid in water and acetonitrile as mobile phases A and B, with the flow rate at 0.3 mL/min for 12 min and the column temperature at 40 °C. Separation was conducted under the following conditions: 0–1 min, 5% B; 1–2 min, 5–10% B; 2–6 min, 10–35% B; 6–8.5 min, 35–100% B; 8.5–9.5 min, 100% B; 9.5–10 min, 100–5% B; 10–12 min, 5% B. The MS analysis was conducted using the Q-Orbitrap mass spectrometer (Thermo Scientific, USA) with electrospray ionization (ESI), operating in the negative ionization full scan mode. The flow rates of auxiliary gas and sheath gas were 10 and 45 (arbitrary units), respectively. The auxiliary gas heater temperature was 300 °C. The capillary temperature was 320 °C. The spray voltage was 3.1 kV and the S-lens RF level was 50 V. The normalized collision energy (NCE) was 30 eV. The resolution of full scan and ddMS2 were 70,000 and 35000, respectively. The full MS scan ranges were set from 66.7 to 1000 *m/z*. Data were acquired and processed using ThermoXcalibur 3.0 software (Thermo Scientific, USA).

Tentative identification of non-volatiles was based on the comparison of retention time, *m/z* values and MS/MS fragments with standards or data from databases (e.g. Massbank and MzCloud) when standards were unavailable. For non-volatiles with available standards, quantitation was determined using a corresponding calibration curve. For non-volatiles without available standards, relative quantitation was calculated by comparing the relative intensities of the parent ions between samples.

2.4. Determination of volatile components

The volatile components were determined using headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC–MS), according to a previously published method (Xu, Chen, Du, & Yin, 2017).

Briefly, the fiber of the SPME needle (Supelco, Bellefonte, PA, USA) was heated at 250 °C for 10 min to remove remaining volatiles before each extraction. Thirty milliliters of the infusion containing ethyl caprate as the internal standard was added into a 50 mL glass vial. Then the glass vial was sealed and incubated at 60 °C. The SPME needle was inserted into the glass vial through the cap to absorb volatiles for 60 min. Later, the SPME needle was inserted into the injection port of GC and volatiles were desorbed at 250 °C for 5 min.

Volatiles were analyzed using an Agilent6890 gas chromatograph coupled with an Agilent HP 5973 MSD (mass selective detector) (Agilent, Wilmington, DE, USA). The separation was performed on a DB-5MS capillary column (30 m × 250 μm × 0.25 μm). The GC condition was as follows: the GC inlet temperature of 250 °C, and the split ratio of 15:1, the carrier gas (high purity helium) flow of 1.0 mL/min. The separation was conducted under the following conditions: 0–2 min, 40 °C; 2–24.5 min, 40–85 °C; 24.5–26.5 min, 85 °C; 26.5–64.5 min, 85–180 °C; 64.5–66.5 min, 180 °C; 66.5–71.5 min, 180–230 °C; 71.5–73.5 min, 230 °C.

For MS analysis, the temperature of the ion source was 230 °C, the voltage was 70 eV and the scan range was 40 to 400 *m/z*.

Tentative identification of volatiles was made by comparing the MS fragmentation patterns with data from the National Institute for Standards and Technology database (NIST 08, match percentage > 80%). The relative abundance of each compound was calculated by comparing the peak area of each compound to the total peak area.

2.5. Sensory evaluation

The general color, taste and aroma properties of samples were assessed according to the national standard GB/T 21733-2008, by a team of seven qualified panelists (three men and four women, 25–50 years old), all of whom achieved certificates for tea-quality evaluation from the Tea Scientific Society of China. The intensities of taste attributes, including bitterness, astringency and sweet aftertaste, were scored (Yin et al., 2014). Scores ranging from 8 to 10 mean “extremely strong”, 6–8 mean “strong”, 4–6 mean “neutral”, 2–4 mean “weak” and “0–2” mean “extremely weak”. Each evaluation was replicated three times on different days with a randomized order of samples for each test.

The color analysis was further determined by a spectrophotometer (Konica Minolta, CM-3500d). The results were presented as the CIE L*a*b* color space parameters.

2.6. Measurement of antioxidant activities

Hydroxyl radical scavenging activity, superoxide anion scavenging activity and total antioxidant capacity (*T*-AOC) were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute. DPPH radical scavenging activity was carried out according to a previously published method (Sui, Dong, & Zhou, 2014).

2.7. Measurement of anti-inflammatory activities

LPS-stimulated RAW 264.7 cells were used for evaluating the inflammatory activities. Briefly, mouse macrophage RAW264.7 cells were seeded in 96-well plates and incubated in a 37 °C chamber with 5% CO_2 overnight. Then cells were treated with 500 ng/mL LPS and different concentrations of CLB or CLM. Cells treated with vehicle were used as the negative control group. After 24 h treatment, the cell culture supernatant was collected for the determination of NO, PGE2 and TNF-α. The NO, PGE2, and TNF-α secretion were measured using the NO assay kit (Beyotime Biotechnology, Haimen, China), PGE2 ELISA kit (Jiangsu Meimian industrial Co., Ltd., Zhangjiagang, China), and TNF-α ELISA kit (Jiangsu Meimian industrial Co., Ltd., Zhangjiagang, China), respectively. The detailed procedures were described previously (Gao et al., 2019).

2.8. Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). All experiments were carried out in triplicate and repeated in three independent sets of experiments. The results were analyzed with SPSS Version 18.0 for Windows using the one-way analysis of variance with 2-sided Dunnett's post hoc test to determine overall differences between groups. P-values < 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Differences in non-volatile chemical composition

To investigate the differences in non-volatiles between the two infusions, traditional analytical methods were used to determine the differences in the main classes of compounds and modern analytical methods were further applied to figure out the differences in compounds.

General chemical analysis unveiled that polyphenols, soluble sugars and saponins were the predominant components in both infusions (Table 1). The concentrations of these components in the two infusions were much different. The concentration of polyphenols in the *Castanopsis lamontii* bud infusion (CLB) was about 1.5 times higher than that in the *Castanopsis lamontii* mature leaf infusion (CLM). HPLC analysis revealed that over 50% of the total polyphenols were made up of epicatechin, procyanidin B2 and catechin (Table 1). Consistent with the results of the determination of total polyphenols, the concentrations of these three polyphenols in CLM were much lower than those in CLB, respectively. The concentrations of saponins, free amino acids and soluble sugars were also lower in CLM, while the concentrations of soluble polysaccharides and flavones were higher.

Table 1
Main non-volatile classes of compounds in CLB and CLM (mg/mL).

Classes of compounds	CLB	CLM
Polyphenols	2.691 ± 0.078	1.039 ± 0.046*
Soluble sugars	1.187 ± 0.123	0.931 ± 0.017*
Saponins	0.509 ± 0.011	0.426 ± 0.047*
Free amino acids	0.161 ± 0.003	0.122 ± 0.000*
Soluble Proteins	0.089 ± 0.000	0.089 ± 0.005
Soluble polysaccharides	0.047 ± 0.001	0.161 ± 0.005*
Flavones	0.021 ± 0.001	0.032 ± 0.002*
Epicatechin (EC)	1.339 ± 0.008	0.345 ± 0.015*
Procyanidin B2 (PB2)	0.345 ± 0.004	0.159 ± 0.014*
Catechin	0.082 ± 0.002	0.018 ± 0.001*

* Indicates $p < 0.05$. CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

As little information of the compounds in CLB and CLM could be found, non-target screening with high-resolution mass spectrometry was applied for compounds detection. UPLC-tandem MS is a powerful method for qualitative analysis of chemical components in complex samples. According to general chemical analysis, the major components in CLB and CLM belonged to polyphenols. Therefore, negative ion mode, which was more sensitive and suitable than positive ion mode for screening polyphenols (Che et al., 2016), was used in this study. The total ion chromatograms of CLB and CLM resembled (Fig. S1), implying that the non-volatile chemical components of CLB and CLM were similar. A total of 23 compounds were tentatively identified (Table 2). Among them, 5 compounds belong to organic acids, 1 compound belongs to nucleosides, 4 compounds belong to flavan-3-ol monomers, 4 compounds belong to flavan-3-ol dimers, 3 compounds belong to flavones and 6 compounds belong to triterpenoids. Quinic acid, malic acid, citric acid, succinic acid and 3-hydroxy-3-methylglutaric acid were detected organic acids in CLB and CLM. Besides epicatechin and catechin, another two flavan-3-ol monomers, gallocatechin and epigallocatechin were also observed. Procyanidins, other than procyandin B2, including two procyandin B2 isomers and one (E)C-(E)GC dimer, were detected. Chrysoeriol and 4′-methylchrysoeriol were identified as representative flavones in CLB and CLM. In addition, various triterpenoid saponin-O-hexosides and triterpenoids were detected, which were in accordance with the general chemical analysis. Since triterpenoids usually had multiple stereoisomers and positional isomers, we only could assure that the major triterpenoids were madecassic acid isomer and asiatic acid isomer. Further experiments are needed to get more detailed information about these triterpenoids and their hexosides.

Although CLB and CLM shared similar non-volatile compounds, the concentrations were quite different. We measured the exact concentrations of identified components with corresponding standards and relative abundances of tentative components by comparing the relative intensity of the representative peak of each component. Compared with CLB, the levels of most compounds, including 8 flavan-3-ol monomers and dimers, 6 triterpenoid saponin-O-hexosides and triterpenoids, 3 flavones, uridine, citric acid and 3-hydroxy-3-methylglutaric acid, were significantly reduced in CLM. The exceptions were three organic acids. Among them, the level of quinic acid, the most abundant organic acid in CLB and CLM, was higher in CLM. Meanwhile, the levels of malic acid and succinic acid were not significantly different between the two infusions.

In a word, the non-volatile compounds in CLB and CLM were similar, but their concentrations were different. As many non-volatiles in CLB and CLM were known to be taste compounds and/ or active compounds, the distinguishable concentrations of these compounds between CLB and CLM indicated that CLB and CLM might possess different sensory properties and bioactivities.

3.2. Differences in volatile chemical composition

To investigate the differences in volatiles between the two infusions, the GC–MS analysis was carried out. Thirty-four aroma compounds were detected in CLB and twenty in CLM (Fig. 1 & Table 3). Among these, fifteen aroma compounds were found in both infusions. Nineteen volatiles were only detected in CLB and five only in CLM. Aldehydes, alcohols and ketones were the key classes of volatiles in CLB and CLM. In CLB, 12 aldehydes, 7 alcohols, 5 ketones, 3 esters, 2 alkenes, 2 alkanes and 3 other types of volatiles were detected. In CLM, 10 aldehydes, 2 alcohols, 5 ketones, 1 ester, 1 alkane and 1 other type of volatiles were observed.

Table 2
Identification of constituents in CLB and CLM by UPLC-QE-Orbitrap-MS.

No.	RT (min)	Molecular formula	[M−H]-		MS/MS Fragments *m/z*	Tentative identification	Concentration (μg/mL)	
			Theoretical	Experimental			CLB	CLM
1	0.68	unknown	unknown	272.9577	158.9777; 130.9830	unknown		74.77 ± 1.94(%)*
2	0.84	$C_7H_{12}O_6$	191.0550	191.0551	85.0291	Quinic acid	202.64 ± 6.70	340.02 ± 3.08*
3	0.96	$C_4H_6O_5$	133.0131	133.0136	115.0031; 89.0240; 71.0135	Malic acid	19.23 ± 0.11	19.88 ± 0.50
4	1.19	$C_6H_8O_7$	191.0186	191.0188	111.0083; 87.0083; 85.0291	Citric acid	12.28 ± 0.24	8.10 ± 0.09*
5	1.24	$C_9H_{12}N_2O_6$	243.0612	243.0610	200.0555; 152.0346; 110.0243	Uridine		36.33 ± 2.22(%)*
6	1.45	$C_4H_6O_4$	117.0182	117.0188	99.0083; 73.0291	Succinic acid	2.54 ± 0.01	2.97 ± 0.41
7	1.58	$C_6H_{10}O_5$	161.0444	161.0448	143.0343; 99.0447; 57.0343	3-Hydroxy-3-methylglutaric acid		21.99 ± 4.80(%)*
8	3.45	$C_{15}H_{14}O_7$	305.0656	305.0653	219.0651; 179.0340; 125.0237	Gallocatechin	6.87 ± 0.15	2.31 ± 0.00*
9	4.18	$C_{30}H_{26}O_{13}$	593.1290	593.1279	407.0753; 289.0704; 125.0238	(*Epi*)gallocatechin-(*Epi*)catechin dimer		27.61 ± 0.26(%)*
10	4.53	$C_{15}H_{14}O_7$	305.0656	305.0652	219.0651; 179.0340; 125.0237	Epigallocatechin	15.80 ± 0.09	7.03 ± 0.25*
11	4.84	$C_{15}H_{14}O_6$	289.0707	289.0703	245.0806; 203.0704; 125.0238	Catechin	78.31 ± 1.19	16.24 ± 0.35*
12	5.07	$C_{30}H_{26}O_{12}$	577.1341	577.1324	407.0754; 289.0705; 125.0238	Procyanidin B2	340.83 ± 5.71	147.52 ± 2.63*
13	5.26	$C_{30}H_{26}O_{12}$	577.1341	577.1324	439.1014; 289.0705; 165.0184	Procyanidin B2 isomer		41.66 ± 2.63(%)*
14	5.36	$C_{15}H_{14}O_6$	289.0707	289.0702	245.0806; 203.0704; 125.0238	Epicatechin	1335.12 ± 2.22	340.85 ± 0.99*
15	6.17	$C_{30}H_{26}O_{12}$	577.1341	577.1329	439.1013; 329.0652; 125.0238	Procyanidin B2 isomer		38.93 ± 0.57(%)*
16	6.49	$C_{24}H_{22}O_{15}$	549.0875	549.0863	505.0970; 463.0865; 300.0262	Quercetin-3-(6′′-malonyl)-Glucoside		71.26 ± 1.76(%)*
17	7.22	$C_{36}H_{58}O_{12}$	681.3844	711.3882 [M + HCOO]-	519.3301; 457.3302; 407.2936	Triterpenoid saponin-O-hexoside		46.89 ± 1.17(%)*
18	7.44	$C_{36}H_{58}O_{11}$	665.3895	711.3928 [M + HCOO]-	503.3359; 490.3236; 453.2983	Triterpenoid saponin-O-hexoside		57.90 ± 2.25(%)*
19	7.91	$C_{36}H_{58}O_{11}$	665.3895	711.3931 [M + HCOO]-	503.3360; 485.3253; 441.3356	Triterpenoid saponin-O-hexoside		62.33 ± 2.24(%)*
20	8.21	$C_{36}H_{58}O_{10}$	649.3946	695.3840 [M + HCOO]-	487.3409; 469.3303	Triterpenoid saponin-O-hexoside		56.50 ± 2.06(%)*
21	8.30	$C_{16}H_{12}O_6$	299.0550	299.0545	284.0311; 256.0363	Chrysoeriol	7.63 ± 0.06	5.48 ± 0.21*
22	8.69	$C_{17}H_{14}O_6$	313.0707	313.0704	298.0468; 283.0234; 269.0443	4′-Methylchrysoeriol		63.20 ± 0.09(%)*
23	8.78	$C_{30}H_{48}O_6$	503.3367	503.3358	485.3254; 473.3254; 441.3356	Madecassic acid isomer		43.25 ± 0.29(%)*
24	9.23	$C_{30}H_{48}O_5$	487.3418	487.3409	469.3300; 443.3508	Asiatic acid isomer		12.96 ± 0.77(%)*

* Indicates $p < 0.05$.For components not verified with standards, the relative abundances were calculated by comparing the relative intensity of the representative peak and presented as the percentage of CLB. CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

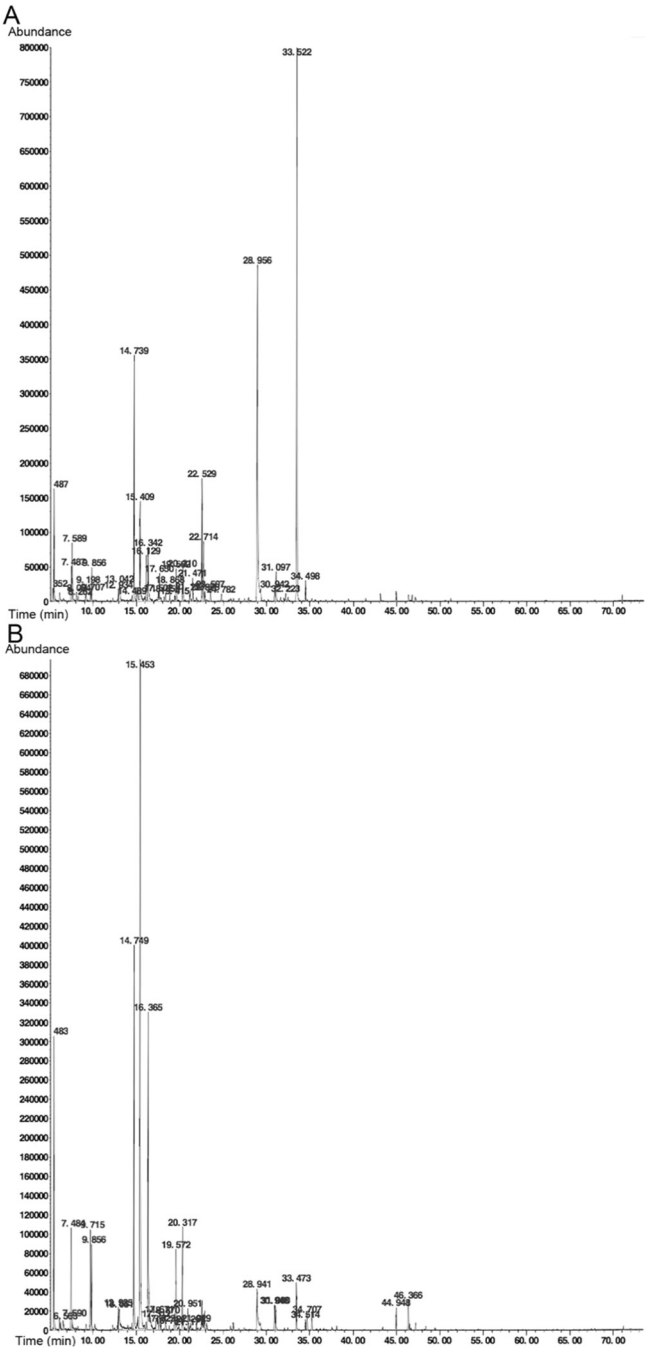

Fig. 1. The GC–MS total ion chromatograms of CLB (A) and CLM (B). CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

Relative quantitation revealed that volatiles in CLB were composed of 35.6% alcohols, 25.4% esters, 17.9% aldehydes, 12.5% ketones, 1.8% alkanes, 1.2% alkenes and 1.9% other types of volatiles. Meanwhile, volatiles in CLM were composed of 56.5% aldehydes, 20.5% ketones, 3.6% esters, 2.4% alcohols, 0.2% alkanes and 0.9% other types of volatiles. Compared with CLB, the relative abundances of alcohols and esters remarkably dropped, and the relative abundances of aldehydes and ketones significantly increased. Among the 15 volatiles which existed in both infusions, 11 were increased, 2 were decreased, and 2 were not significantly altered in CLM compared with CLB. Nerol and methyl salicylate were the predominant aroma compounds in CLB, accounting for about 47% of total volatiles. However, the relative abundances of these two volatiles were dramatically decreased in CLM, accounting for only about 5.2% of total volatiles in CLM. Instead, (E, E)-2,4-heptadienal, 6-methyl-5-hepten-2-one and hexanal became the predominant aroma compounds in CLM, accounting for about 29.0%, 13.8% and 10.3% of total volatiles in CLM, respectively.

The above results showed that the types and proportions of aroma compounds in CLB and CLM were quite distinctive, suggesting CLB and CLM have different aroma characteristics.

3.3. Differences in sensory properties

The results of component analysis revealed that CLB and CLM had different non-volatile and volatile profiles, implying CLB and CLM might have different tastes and scents. Sensory properties are vital for the popularity and quality of daily-consumed beverages. In this study, CLB and CLM were found to have distinguishable color, aroma and taste characteristics.

3.3.1. Differences in color

CLB was golden yellow, while CLM was greenish yellow (Fig. 2B). Color analysis (Fig. 2C) using a spectrophotometer revealed that CLB had higher a value and b value than those of CLM, indicating that CLB contained higher proportions of red and yellow color, presenting a warmer tone than CLM. In contrast, CLM contained higher proportions of green and blue color, presenting a cooler tone. The spectrophotometry data were consistent with the results from visual observation.

3.3.2. Differences in aroma

The aroma of the CLB and CLM belonged to different categories. CLB had a pleasant floral scent, while CLM was rather grassy and monotonous. According to the criteria for classification of *Camellia sinensis* tea grade, there is a negative relationship between the intensity of grassy scent and the grade of tea. Furthermore, tea with a floral aroma tends to have a higher score in tea classification. This indicates that a grassy scent is less popular while a floral scent is more popular among customers. Based on this point, the aroma of CLB was speculated to be more acceptable than the aroma of CLM.

Volatiles are main contributors to scents. The results of GC–MS analysis revealed that the number of volatiles in CLM was much less than that in CLB (20 vs 34). It explained why CLM smelt much more monotonous than CLB.

Compared with CLM, there were more diverse and abundant floral aroma compounds in CLB, determining the pleasant floral scent of CLB. Several floral volatiles, including (Z)-β-ocimene, (E)-furanoid linalool oxide, linalool and nonanal, were only observed in CLB but not in CLM. The total relative abundances of floral volatiles in CLB were about 31.5%. Nerol, which had a sweet natural neroli aroma, itself alone accounted for about 24.0% of total volatiles in CLB and turned out to be the most abundant volatile in CLB. The total relative abundances of floral volatiles sharply dropped to 3.6% in CLM, causing the loss of floral aroma in CLM. The decrease was basically attributed to the decrease of nerol, whose relative abundance was merely 1.7% in CLM. Minty or cooling volatiles were also key volatiles in CLB. Methyl salicylate, a compound with wintergreen mint scent, was the second abundant volatile in CLB, accounting for 23.0% of total volatiles. Eucalyptol, which had a characteristic eucalyptus and camphor scent, was only detected in CLB. Isophorone was another cooling volatile only observed in CLB. The existing of these minty or cooling volatiles together with floral volatiles made CLB smelt floral and fresh. In CLM, only one minty or cooling volatile was found, and it was methyl salicylate. The relative abundance of methyl salicylate in CLM was about 3.6%, much lower than that in CLB. Therefore, CLM hardly smelt fresh and brisk. Besides these, some volatiles with fruity scents or unripe fruity scents, including (E)-2-Hexen-1-ol, 2-heptanone, limonene, (Z)-3-hexenyl valerate and neral, were also observed in CLB, contributing to weak fruity scents in CLB. In addition, earthy and mouldy volatiles

Table 3
Volatile compounds determined by GC–MS.

Retention time	CAS number	Molecular formula	Molecular weight	Name	Relative abundance (%)		Aroma properties
					CLB	CLM	
5.362	141-79-7	$C_6H_{10}O$	98	3-Penten-2-one, 4-methyl-	0.427 ± 0.008*	N.D.	Pungent
5.487	66-25-1	$C_6H_{12}O$	100	Hexanal	4.442 ± 0.692	10.291 ± 2.053*	Green, grassy
7.49	6728-26-3	$C_6H_{10}O$	98	(E)-2-Hexenal	1.232 ± 0.027	3.407 ± 0.126*	Green
7.586	544-12-7	$C_6H_{12}O$	100	3-Hexen-1-ol	2.382 ± 0.326*	N.D.	Green
8.084	928-95-0	$C_6H_{12}O$	100	(E)-2-Hexen-1-ol	0.317 ± 0.056*	N.D.	Fresh green, unripe fruity
8.262	4312-76-9	$C_6H_{14}O_2$	118	Hydroperoxide, hexyl	0.163 ± 0.012*	N.D.	
9.198	110-43-0	$C_7H_{14}O$	114	2-Heptanone	0.607 ± 0.012*	N.D.	Fruity
9.707	6728-31-0	$C_7H_{12}O$	112	(Z)-4-Heptenal	0.351 ± 0.032	3.276 ± 0.124*	Green, creamy
9.858	111-71-7	$C_7H_{14}O$	114	Heptanal	1.311 ± 0.133	3.071 ± 0.418*	Fruity
12.934	57266-86-1	$C_7H_{12}O$	112	(Z)-2-Heptenal	0.483 ± 0.068	0.813 ± 0.088*	
13.042	100-52-7	C_7H_6O	106	Benzaldehyde	1.058 ± 0.051	1.181 ± 0.257	Strong sharp almond aroma
14.489	3391-86-4	$C_8H_{16}O$	128	1-Octen-3-ol	0.260 ± 0.014*	N.D.	Mushroom aroma, earthy
14.741	110-93-0	$C_8H_{14}O$	126	6-Methyl-5-hepten-2-one	9.173 ± 0.412	13.794 ± 0.190*	Citrus and lemongrass aroma
15.411	4313-03-5	$C_7H_{10}O$	110	(E, E)-2,4-Heptadienal	5.318 ± 1.166	28.993 ± 1.412*	Fatty, green, oily
16.134	72237-36-6	$C_8H_{14}O_2$	142	4-Hexen-1-ol, acetate	1.963 ± 0.306*	N.D.	
17.502	138-86-3	$C_{10}H_{16}$	136	Limonene	0.394 ± 0.156*	N.D.	Lemon, citrus aroma
17.652	470-82-6	$C_{10}H_{18}O$	154	Eucalyptol	1.175 ± 0.133*	N.D.	Eucalyptus, camphor aroma
17.671	104-76-7	$C_8H_{18}O$	130	2-Ethyl-1-hexanol	N.D.	0.745 ± 0.074*	Citrus aroma
18.359	122-78-1	C_8H_8O	120	Benzeneacetaldehyde	0.322 ± 0.012	0.528 ± 0.068*	Sweet floral, hyacinth aroma
18.791	16747-50-5	C_8H_{16}	116	Cyclopentane, 1-ethyl-1-methyl-	N.D.	0.171 ± 0.020*	
18.868	3338-55-4	$C_{10}H_{16}$	136	(Z)-β-Ocimene	0.870 ± 0.259*	N.D.	Warm floral
19.415	78-59-1	$C_9H_{14}O$	138	Isophorone	0.234 ± 0.002*	N.D.	Cooling woody
19.562	2548-87-0	$C_8H_{14}O$	126	(E)-2-Octenal	1.499 ± 0.097	3.832 ± 0.477*	Fresh cucumber aroma
20.312	38284-27-4	$C_8H_{12}O$	124	3,5-Octadien-2-one	2.033 ± 0.064	4.343 ± 0.316*	Fruity, fatty, mushroom aroma
21.471	34995-77-2	$C_{10}H_{18}O_2$	170	(E)-Furanoid linalool oxide	0.920 ± 0.004*	N.D.	Floral
22.531	78-70-6	$C_{10}H_{18}O$	154	Linalool	5.144 ± 0.028*	N.D.	Floral
22.716	29957-43-5	$C_{10}H_{16}O$	152	Dehydrolinalool	2.556 ± 0.110*	N.D.	Mouldy
22.876	124-19-6	$C_9H_{18}O$	142	Nonanal	0.497 ± 0.028*	N.D.	Waxy, rosy, orange peel aroma
23.507	95452-08-7	$C_{11}H_{18}$	150	Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-	0.655 ± 0.177*	N.D.	
24.782	140-29-4	C_8H_7N	117	Benzyl nitrile	0.368 ± 0.045*	N.D.	
28.956	119-36-8	$C_8H_8O_3$	152	Methyl salicylate	23.028 ± 0.228	3.564 ± 0.181*	Wintergreen mint
30.942	432-25-7	$C_{10}H_{16}O$	152	β-Cyclocitral	0.603 ± 0.001	1.092 ± 0.006*	Woody
31.099	496-16-2	C_8H_8O	120	2, 3-Dihydrobenzofuran	1.382 ± 0.071	0.953 ± 0.306	
32.223	35852-46-1	$C_{11}H_{20}O_2$	184	(Z)-3-Hexenyl valerate	0.391 ± 0.097*	N.D.	Green fruity
33.524	106-25-2	$C_{10}H_{18}O$	154	Nerol	24.035 ± 3.926*	1.698 ± 0.341	Sweet natural neroli aroma
34.507	141-27-5	$C_{10}H_{16}O$	152	Neral	0.786 ± 0.064*	N.D.	Lemon aroma
44.948	3879-26-3	$C_{13}H_{22}O$	194	Nerylacetone	N.D.	0.896 ± 0.213*	Woody
46.366	14901-07-6	$C_{13}H_{20}O$	192	β-Ionone	N.D.	1.353 ± 0.478*	Floral
49.476	1888-57-9	$C_8H_{16}O$	128	3-Hexanone, 2,5-dimethyl-	N.D.	0.106 ± 0.066*	

* Indicates $p < 0.05$.N.D. is short for not detected. CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

(e.g., 1-octen-3-ol and dehydrolinalool) enriched the scents of CLB.

Unlike CLB, green and grassy volatiles predominated in the scent of CLM, endowing the grassy scent of CLM. Although the variety of green and grassy volatiles in CLM didn't significantly differ from CLB, the relative abundances of them remarkably increased. The total relative abundance of green and grassy volatiles in CLM was almost two times higher than that in CLB (46.0% vs 14.4%). The top three abundant volatiles in CLM were (E, E)-2,4-heptadienal, 6-methyl-5-hepten-2-one and hexanal. All of them smelt green or grassy. And each of them had higher relative abundance in CLM. (E)-2-hexenal and (Z)-4-heptenal, two other green volatiles, were also found increased in CLM. Besides, the relative abundances of two woody volatiles (β-cyclocitral and nerylacetone) and four fruity volatiles (heptanal, 2-ethyl-1-hexanol, (E)-2-octenal, 3,5-octadien-2-one) were higher in CLM than those in CLB, respectively. The mixture of these volatiles formed a typical scent of green plants.

3.3.3. Differences in taste

The taste of CLB and CLM also differed (Fig. 2D). CLB tasted bitter at first, but gave way to a strong and long-lasting sweet aftertaste. In contrast, CLM tasted astringent and had a weak sweet aftertaste. According to the criteria for classification of *Camellia sinensis* tea grade, both bitterness and astringency can be unpleasant, but a sweet aftertaste is appreciated by customers and is usually positively correlated with the quality of *Camellia sinensis* tea. Therefore, the taste of CLB was thought to be better acceptable than CLM.

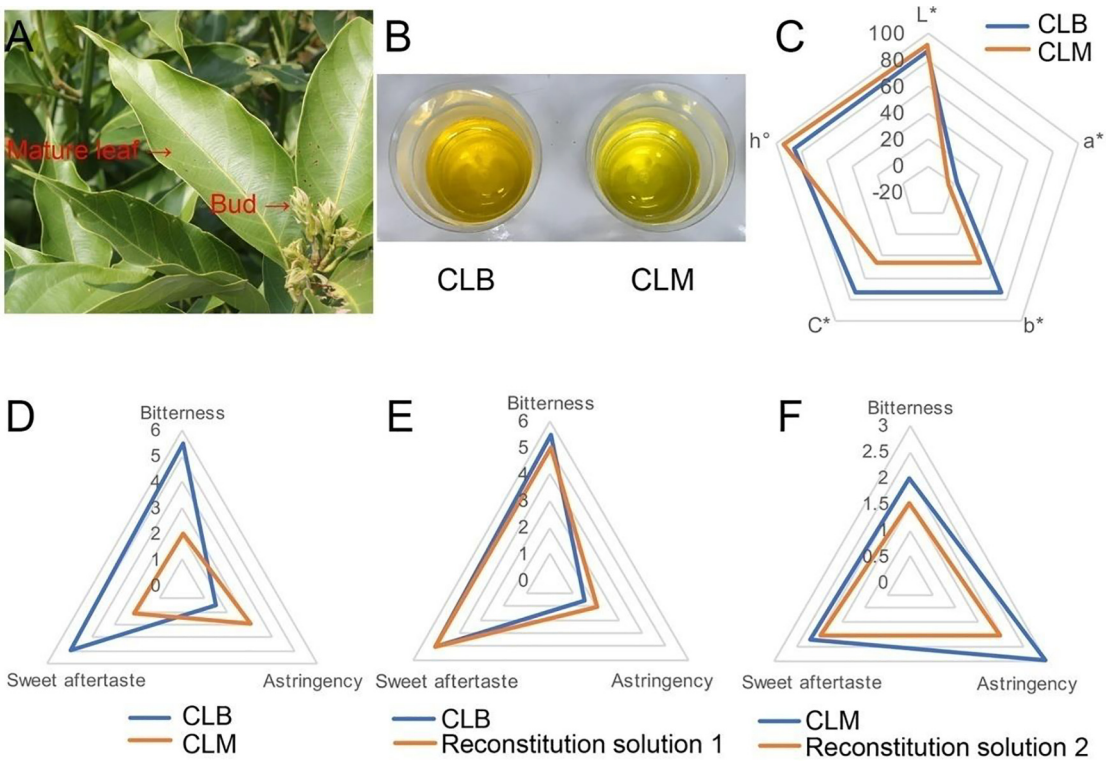

Fig. 2. Color and taste assessment of CLB and CLM. A, photo of *Castanopsis lamontii* buds and mature leaves; B, photo of CLB and CLM; C, color analysis determined by a spectrophotometer; D, taste scores of CLB and CLM; E, taste scores of CLB and Reconstitution solution 1 (containing identical concentrations of epicatechin and procyanidin B2 to CLB); F, taste scores of CLM and Reconstitution solution 2 (containing identical concentrations of epicatechin and procyanidin B2 to CLM). CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

A previous research has implicated catechins as key taste compounds, contributing to the bitterness and astringency in green tea infusions (Xu et al., 2018). Certain catechins even have a sweet aftertaste (Zhang et al., 2016). Catechin monomers, including epicatechin and catechin, are rated as more bitter than astringent (Drewnowski & Gomez-Carneros, 2000). Compared with its stereoisomer of catechin, epicatechin has a lower taste threshold for bitterness and a higher taste threshold for astringency (Gacon, Peleg, & Noble, 1996). As the degree of polymerization increases, catechins oligomers and polymers become progressively more astringent and less bitter (Gacon et al., 1996). For example, procyanidin B2 (i.e., (−)-epicatechin-(4β → 8)-(−)-epicatechin), a dimer of epicatechin, tastes astringent rather than bitter (Gonzalo-Diago, Dizy, & Fernandez-Zurbano, 2014; Hernandez, Song, & Menendez, 2017). To evaluate the contributions of epicatechin, procyanidin B2 and catechin to the taste of CLB, the dose-over-threshold factors of each compound were calculated (Table S1). Based on the results, epicatechin had larger contributions to bitterness and astringency and was the principal bitter compound in CLB. Procyanidin B2 contributed more to astringency than bitterness. Catechin, although confirmed as bitter and astringent, had limited influence on the taste because its concentration in CLB was below threshold. To further investigate the role of epicatechin and procyanidin B2 in the taste of CLB, a reconstitution solution (Reconstitution solution 1) containing 1.339 mg/mL epicatechin and 0.345 mg/mL procyanidin B2 (identical to the concentrations found in CLB) was prepared and tasted by panelists. The intensities of bitterness, astringency and sweet aftertaste of this reconstitution solution were deemed similar to those of CLB, suggesting that epicatechin and procyanidinB2 were the principal taste compounds (Fig. 2E).

Compared with CLB, the concentrations of epicatechin and procyanidin B2 were much lower in CLM, which markedly influenced the taste. The intensities of bitterness, astringency and sweet aftertaste were much weaker in CLM. However, the decrease of astringency intensity was less than those of bitterness and sweet aftertaste, making astringency the predominant flavor of CLM. A reconstitution solution (Reconstitution solution 2) containing 0.345 mg/mL epicatechin and 0.159 mg/mL procyanidin B2 had a similar taste profile to that of CLM in terms of bitterness and sweet aftertaste, but less astringency (Fig. 2F). This result demonstrated that epicatechin and procyanidin B2 were essential for the taste of CLM. The inconsistency between the astringency of CLM and Reconstitution solution 2 suggested that there might be other astringent compounds in CLM. Based on the results of chemical composition analysis, CLM contained higher concentrations of flavones and quinic acid than CLB. Many flavones and flavone glycosides have been shown to be astringent even at very low concentrations (Scharbert, Holzmann, & Hofmann, 2004), and quinic acid was identified to be responsible for the astringent taste of roasted coffee (Buffo & Cardelli-Freire, 2004). Besides, the inconsistency could be caused by the interactions between taste compounds and other chemicals. Previous studies have found that the flavor characteristics of taste compounds could be influenced by certain ions and other taste compounds. For example, the presence of Ca^{2+} and alkaloids can enhance the astringent taste of catechins (Yin et al., 2014).

3.4. Differences in bioactivities

3.4.1. Differences in antioxidant capacities

Our previous study demonstrated that CLB potently prevented oxidative stress-induced cell damage and scavenged free radicals *in vitro* (Gao et al., 2019). In this study, the antioxidant capacities of CLB and CLM were assessed. Compared with CLM, CLB displayed stronger activities in scavenging hydroxyl free radical, superior superoxide anion and DPPH radical (Fig. 3A–C). Although the concentrations of polyphenols in CLM were less than half of CLB, the *T*-AOC was higher (Fig. 3D).

To our knowledge, epicatechin and procyanidin B2, although

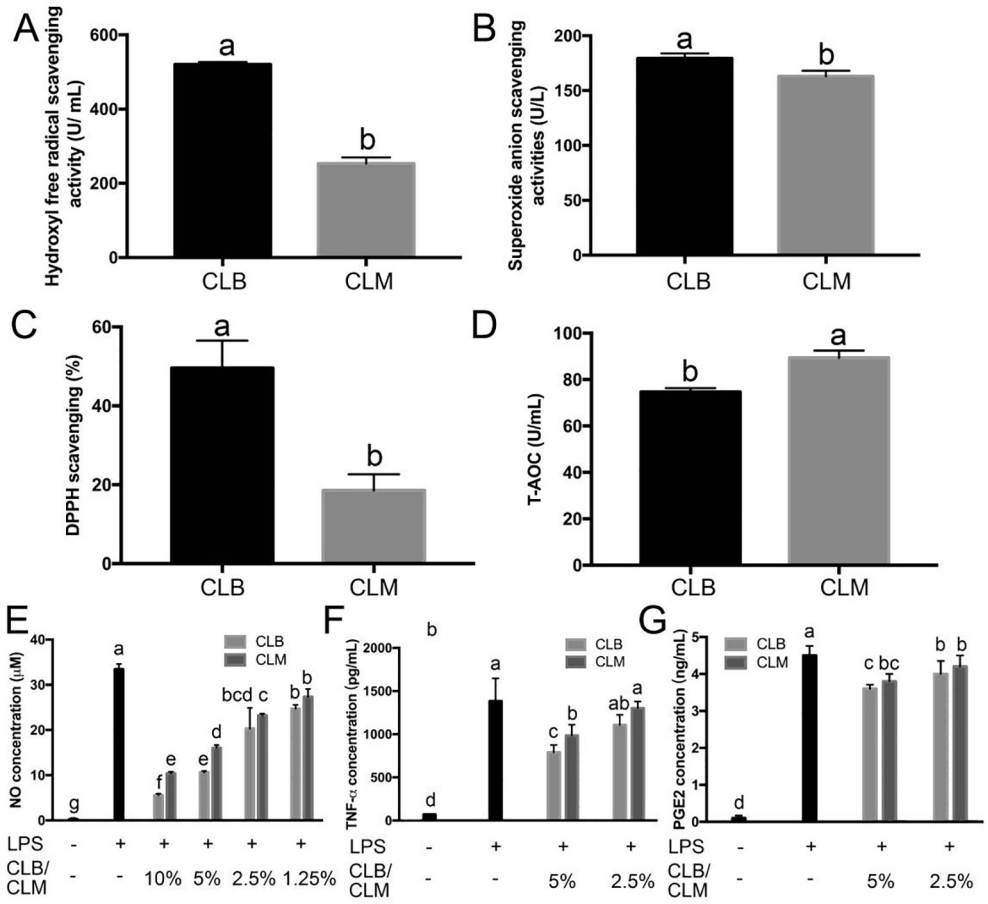

Fig. 3. Bioactivity assessment of CLB and CLM. A, hydroxyl free radical scavenging activity; B, superoxide anion scavenging activity; C, DPPH scavenging activity; D, total antioxidant capacity. E–G, Effects of CLB and CLM on suppressing lipopolysaccharide (LPS)-induced secretion of nitric oxide (NO) (E), prostaglandin E2 (PGE2) (F), tumor necrosis factor α (TNF-α) (G) of RAW 264.7 cells. The same letter within each column indicates no significant difference ($p > 0.05$). CLB, *Castanopsis lamontii* bud infusion; CLM, *Castanopsis lamontii* mature leaf infusion.

important, are not the only antioxidant compounds in CLB. Polyphenols excluding epicatechin and procyanidin B2, saponins, flavones and soluble polysaccharides are all potential antioxidant candidates. Kim et al. (2018) found that catechin, epicatechin and taxifolin, as well as some volatile compounds, were active antioxidant components of *Quercusacuta* Thunb. (*Fagaceae*) extracts. Zhao et al. (2011) found that there was a positive linear correlation between antioxidant activity and total tannin content in extracts and fractions of *Castaneamo llissima* Blume, and hydrolysable tannins were characterized as the predominant antioxidant components. Tahmouzi (2014) confirmed that polysaccharides from Zagros oak (*Quercusbrantii* Lindl) leaf had strong scavenging activities *in vitro* on DPPH and hydroxyl radicals. In addition, a previous study found that organic acids, flavones and flavone glycosides, such as quinic acid, quercetin, kaempferol, quercetin derivatives and kaempferol derivatives were positively correlated with antioxidant activity of the *Fagaceae* family (Lee et al., 2015). Therefore, it was speculated that the stronger *T*-AOC of CLM might be attributed to higher concentrations of flavones and soluble polysaccharides. However, further studies are needed to verify this hypothesis.

3.4.2. Differences in anti-inflammatory activity

Shaji herbal tea, the processed product of *Castanopsis lamontii* buds, is believed to have preventive effects on oral inflammatory diseases, such as periodontitis and pharyngitis. Most oral inflammatory diseases are usually caused by bacterial infection and result in pain, swelling, redness and even loss of function (Nam, 1989; Suzuki, Yoneda, & Hirofuji, 2013). LPS, a key component of the outer membrane of Gram-negative bacteria, is a potent stimulator of inflammation (Smirnova, Guo, Birchall, & Pearson, 2003). LPS can bind to toll-like receptor 4, leading to the nuclear translocation of a transcription factor called nuclear factor-kappa B (NF-κB) (Lu, Yeh, & Ohashi, 2008). The NF-κB protein mediates the inducible transcription of various pro-inflammatory factors (e. g., TNF-α and Interleukin-6), as well as several enzymes which synthesize inflammatory mediators (Wang, Xiang, Cui, Lin, & Zhang, 2012; Yao et al., 2018). For example, cyclooxygenase-2 (COX-2), an enzyme generates pro-inflammatory prostaglandins (PGEs), is up-regulated by the activation of NF-κB. Inducible nitric oxide synthase (iNOS), an enzyme produces nitric oxide (NO), is also a downstream protein of NF-κB.

In this research, macrophage RAW 264.7 cells were incubated with LPS to cause inflammatory responses. As shown in Fig. 3E–G, the levels of NO, TNF-α and PGE2 in the model group (where cells were only treated with LPS) were significantly elevated compared with the negative control group, suggesting the inflammation was successfully induced. When co-treated with CLB or CLM, the levels of NO, TNF-α and PGE2 were decreased, indicating that both CLB and CLM displayed anti-inflammatory activities. Besides, the anti-inflammatory activities of CLB and CLM were dose-dependent. Compared with CLM, the anti-inflammatory activity of CLB was stronger, especially at high concentrations. Previously, PB2 was identified as the major anti-inflammatory compound in CLB (Gao et al., 2019). Epicatechin also contributed to the anti-inflammatory effect of CLB, but much weaker than PB2. CLB contained higher levels of PB2 and epicatechin. Therefore, it was not surprising that CLB exhibited stronger anti-inflammatory activity.

4. Conclusion

In this study, the chemical composition, sensory properties and bioactivities of CLB and CLM were assessed and compared. In general, CLB and CLM shared similar non-volatile components, but in different concentrations. CLB contained more polyphenols and less quinic acid

than CLM. The volatiles in CLB and CLM were distinctive. CLB contained more diverse and abundant floral and fresh aroma compounds (e.g., nerol and methyl salicylate), while CLM contained more green and grassy aroma compounds (e.g., hexanal). Different chemical compositions lead to different sensory quality and bioactivities. The overall acceptability of CLB in sensory quality was higher than CLM, as CLB showed more pleasant aroma and taste. CLB was more capable in scavenging hydroxyl free radical, superoxide anion and DPPH radical. And CLB was more potent in inhibiting LPS-induced inflammatory response in RAW 264.7 cells. These findings enhance our understanding of the differences between *Shaji* herbal teas made of different raw materials and provide guidance for selecting appropriate materials to make good-quality *Shaji* herbal tea.

Author contributions

Ying Gao, Yong-Quan Xu and Jun-Feng Yin designed the study. Ying Gao, Jie-Qiong Wang and Yan-Qing Fu performed the research including data analysis and literature search. Ying Gao and Yong-Quan Xu wrote and revised the manuscript. John Shi revised the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126370.

References

Buffo, R. A., & Cardelli-Freire, C. (2004). Coffee flavour: An overview. *Flavour and Fragrance Journal, 19*(2), 99–104.

Che, Y. Y., Wang, Z. B., Zhu, Z. Y., Ma, Y. Y., Zhang, Y. Q., Gu, W., ... Rao, G. X. (2016). Simultaneous Qualitation and quantitation of chlorogenic acids in kuding tea using ultra-high-performance liquid chromatography-diode array detection coupled with linear ion trap-orbitrap mass spectrometer. *Molecules, 21*(12), 1728.

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: A review. *American Journal of Clinical Nutrition, 72*(6), 1424–1435.

Gacon, K., Peleg, H., & Noble, A. C. (1996). Bitterness and astringency of flavan-3-OL monomers, dimers and trimers. *Food Quality and Preference, 7*(3–4), 343–344.

Gao, Y., Zhang, X., Yin, J., Du, Q., Tu, Y., Shi, J., & Xu, Y. (2019). Castanopsis lamontii water extract shows potential in suppressing pathogens, lipopolysaccharide-induced inflammation and oxidative stress-induced cell injury. *Molecules, 24*(2), 273.

Gonzalo-Diago, A., Dizy, M., & Fernandez-Zurbano, P. (2014). Contribution of low molecular weight phenols to bitter taste and mouthfeel properties in red wines. *Food Chemistry, 154*, 187–198.

Hernandez, M. M., Song, S., & Menendez, C. M. (2017). Influence of genetic and vintage factors in flavan-3-ol composition of grape seeds of a segregating Vitis vinifera population. *Journal of the Science of Food and Agriculture, 97*(1), 236–243.

Hu, F., Lou, D. W., Zhu, B., Lian, L. L., Ren, H., Jin, L., ... Yang, Q. L. (2015). HPLC determination of catechin, epicatechin and procyanidin B2 in plant fruits. *Physical Testing and Chemical Analysis Part B: Chemical Analysis, 51*, 489–492.

Khan, M. R., Kihara, M., & Omoloso, A. D. (2001). Antimicrobial activity of Lithocarpus celebicus. *Fitoterapia, 72*(6), 703–705.

Kim, M. H., Park, D. H., Bae, M. S., Song, S. H., Seo, H. J., Han, D. G., ... Cho, S. S. (2018). Analysis of the active constituents and evaluation of the biological effects of quercus acuta Thunb. (Fagaceae) extracts. *Molecules, 23*(7), 1772.

Lee, S., Oh, D. G., Lee, S., Kim, G. R., Lee, J. S., Son, Y. K., ... Lee, C. H. (2015). Chemotaxonomic metabolite profiling of 62 indigenous plant species and its correlation with bioactivities. *Molecules, 20*(11), 19719–19734.

Lu, Y. C., Yeh, W. C., & Ohashi, P. S. (2008). LPS/TLR4 signal transduction pathway. *Cytokine, 42*(2), 145–151.

Nam, I. W. (1989). Clinical studies on oral infections in the oral cavity and maxillofacial regions. *Taehan Chikkwa Uisa Hyophoe Chi, 27*(3), 309–315.

Scharbert, S., Holzmann, N., & Hofmann, T. (2004). Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *Journal of Agricultural and Food Chemistry, 52*(11), 3498–3508.

Smirnova, M. G., Guo, L., Birchall, J. P., & Pearson, J. P. (2003). LPS up-regulates mucin and cytokine mRNA expression and stimulates mucin and cytokine secretion in goblet cells. *Cell Immunology, 221*(1), 42–49.

Sui, X., Dong, X., & Zhou, W. (2014). Combined effect of pH and high temperature on the stability and antioxidant capacity of two anthocyanins in aqueous solution. *Food Chemistry, 163*, 163–170.

Suzuki, N., Yoneda, M., & Hirofuji, T. (2013). Mixed red-complex bacterial infection in periodontitis. *International Dental Journal, 2013*, 587279.

Tahmouzi, S. (2014). Optimization of polysaccharides from Zagros oak leaf using RSM: Antioxidant and antimicrobial activities. *Carbohydrate Polymers, 106*, 238–246.

Wang, Q. S., Xiang, Y., Cui, Y. L., Lin, K. M., & Zhang, X. F. (2012). Dietary blue pigments derived from genipin, attenuate inflammation by inhibiting LPS-induced iNOS and COX-2 expression via the NF-kappaB inactivation. *PLoS One, 7*(3), e34122.

Xu, Y. Q., Chen, J. X., Du, Q. Z., & Yin, J. F. (2017). Improving the quality of fermented black tea juice with oolong tea infusion. *Journal of Food Science and Technology, 54*(12), 3908–3916.

Xu, Y. Q., Zhang, Y. N., Chen, J. X., Wang, F., Du, Q. Z., & Yin, J. F. (2018). Quantitative analyses of the bitterness and astringency of catechins from green tea. *Food Chemistry, 258*, 16–24.

Yadav, A. K., & Tangpu, V. (2007). Antidiarrheal activity of Lithocarpus dealbata and Urena lobata extracts: Therapeutic implications. *Pharmaceutical Biology, 45*(3), 223–229.

Yao, J., Du, X., Chen, S., Shao, Y., Deng, K., Jiang, M., ... Feng, G. (2018). Rv2346c enhances mycobacterial survival within macrophages by inhibiting TNF-alpha and IL-6 production via the p38/miRNA/NF-kappaB pathway. *Emerg Microbes Infect, 7*(1), 158.

Yin, J. F., Zhang, Y. N., Du, Q. Z., Chen, J. X., Yuan, H. B., & Xu, Y. Q. (2014). Effect of Ca^{2+} concentration on the tastes from the main chemicals in green tea infusions. *Food Research International, 62*, 941–946.

Zhang, Y. N., Yin, J. F., Chen, J. X., Wang, F., Du, Q. Z., Jiang, Y. W., & Xu, Y. Q. (2016). Improving the sweet aftertaste of green tea infusion with tannase. *Food Chemistry, 192*, 470–476.

Zhao, S., Liu, J. Y., Chen, S. Y., Shi, L. L., Liu, Y. J., & Ma, C. (2011). Antioxidant potential of polyphenols and tannins from burs of Castanea mollissima Blume. *Molecules, 16*(10), 8590–8600.