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Volatile profiles and antioxidant activity of different cultivars of *Camellia sinensis var. assamica* grown in Thailand

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Abstract

Tea is considered as the most consumed drink in the world containing high antioxidant capacity. In this study, the volatile compounds, the phenolic content, catechins and caffeine including antioxidant activities of 22 *Camellia sinensis var. assamica* (Assam tea) cultivars were investigated. The volatile compounds were investigated by GC-MS. At least forty-five volatile compounds representing 94.99-99.65% of all cultivars were identified. Limonene, trans-linalool oxide, cis-linalool oxide, linalool, and furfural were detected as the major components among these cultivars. Varied ranges were found in all Assam tea cultivars for the contents of phenolics (113.45-245.55 mg gallic acid/g dry weight), total catechins (170.03-355.59 mg/g dry weight), caffeine (0.92-3.40 mg/g dry weight), and antioxidant activities (1418.68-2728.46 µmol Trolox/g dry weight and 1448.98-2864.17 µmol Trolox/g dry weight for DPPH and ABTS assay, respectively). The antioxidant activity was correlated with phenolic compounds such as epigallocatechin gallate, epicatechin gallate, and catechin gallate. The specific differences among Assam tea cultivars are dependent on the tea cultivar and altitude which may play a significant role in breeding Assam tea cultivars in Thailand for providing its potential health benefits.

1. Introduction

Tea produced from *Camellia sinensis* leaves is the second most popular drink throughout the world next to water (Pripdeevech *et al.*, 2017). Tea drinking has been reported to help to reduce the risk of cancer and heart diseases (Almajano *et al.*, 2008). This benefit is the result of various types of bioactive compounds in tea such as polyphenols, caffeine, and terpenes accounting for its pharmaceutical and aroma properties (Kumazawa and Masuda, 2002). Polyphenol compounds in tea, mainly catechins, were detected up to 30-35% of the dry weight of tea leaves consisting of epicatechin, epicatechingallate, epigallocatechin, epigallocatechingallate, catechin, catechingallate, gallocatechin and gallocatechingallate (Pripdeevech and Machan, 2011). Studies have shown that these catechins are reported to possess antioxidant, anti-inflammatory, antibacterial and anti-allergic activities (Afzal *et al.*, 2015). Caffeine has been reported to improve mood, attention, performance, alertness, the speed at which information is processed and reaction time (Astill *et al.*, 2001). In addition, it was associated with reducing the risk of Parkinson's disease (Li *et al.*, 2012). Volatile aroma components in tea were particularly monoterpenes and sesquiterpenes with its derivatives achieving by fermentation processing methods (Pripdeevech and Machan, 2011).

The amount of the bioactive compounds in tea reflected its quality according to several parameters including cultivars, harvest season, age of the plant, climate, environmental and processing conditions as well as storage (Zeng *et al.*, 2016). The tea cultivar is one of the most important factors affecting tea quality, and it also indicates types and relevant bioactive compound of tea. According to their genetics and cultivars, teas are classified into two main types including Chinese tea (*Camellia sinensis var. sinensis*) and Assam tea (*Camellia sinensis var. assamica*). Tea leaves of both types are processed to produce specific types of tea including green, oolong, and black tea.

In Thailand, tea is cultivated mainly in Chiang Rai and Chiang Mai provinces locating in the northern part of the country accounting for 93% of tea production. Commercially tea products in Thailand are obtained

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from both Assam and Chinese cultivar. About 30% of tea products are commercialized in the domestic market, whereas 70% is exported (Theppakorn *et al.*, 2014). In Thailand, Assam tea is cultivated in a larger planting area than Chinese tea (Theppakorn *et al.*, 2014). It is mainly used to produce Miang or fermented tea (Theppakorn *et al.*, 2012). It is indicated a lifestyle staple of people in northern Thailand especially hill tribes. It is used to welcome house guests or in northern style ceremonies and cultures (Phromrukachat *et al.*, 2010). Although the potential of the various bioactive compound of Assam tea is similar to those found in Chinese tea, the price of Miang is lower than those of Chinese tea due to its astringent flavour from tannins and inappropriate taste of the ratio of sugar, fat, and chlorophylls (Reichart *et al.*, 2005).

To our knowledge, there is less literature on the study of phytochemical profiles and the associated antioxidant activities of different Assam tea cultivars grown in the same conditions. It is of interest to know which tea cultivar could potentially be more beneficial in terms of antioxidant activity. Therefore, the objectives for this study were to determine the volatile profiles, antioxidant activities and total phenolic content in 22 Assam tea cultivars grown in the same conditions.

2. Materials and methods

2.1 Plant material

Shoots with a bud and two leaves from 22 cultivars of Assam tea (*C. sinensis var. assamica*) plants were harvested from the tea garden of the Tea and Coffee Institute, Mae Fah Luang University at BoonRod Rai, Chiang Rai province, Thailand in March 2019. The leaves were immediately processed at 220°C to promote inactivation of the endogenous enzymatic reaction prior to rolling for 30 mins using typical manufacturing approaches from BoonRod Rai. They were stored in the dry place at room temperature until analysis. Assam tea samples obtained from 22 cultivars are listed in Table 1.

Table 1. List of 22 Assam tea cultivars

No.	Province	Location	Altitude (m)	Abbreviation
1	Changrai	Maechan	1058	CR1
2	Changrai	Maechan	853	CR2
3	Changrai	Maung	1012	CR3
4	Changrai	Mae Suai	563	CR4
5	Changrai	Pan	450	CR5
6	Changrai	Thoeng	420	CR6
7	Changrai	Maefahluang	1311	CR7
8	Lampang	Maungpan	1161	LP1
9	Lampang	Ngaao	800	LP2
10	Nan	Muang	737	NN1
11	Nan	Pua	1452	NN2
12	Chiangmai	Omkoi	855	CM1
13	Chiangmai	Chiangdao	1264	CM2
14	Chiangmai	Maetaeng	751	CM3
15	Chiangmai	Chiangdao	1253	CM4
16	Chiangmai	Maetaeng	700	CM5
17	Chiangmai	Maetaeng	712	CM6
18	Chiangmai	Maetaeng	716	CM7
19	Chiangmai	Doisaket	536	CM8
20	Chiangmai	Doisaket	521	CM9
21	Chiangmai	Doisaket	502	CM10
22	Chiangmai	Doisaket	548	CM11

2.2 Chemicals

HPLC-grade acetonitrile, methanol and trifluoroacetic acid were obtained from Merck (Darmstadt, Germany). Water was prepared using an ultrapure water system. The following standards were purchased from Sigma Chemical Co. (Thailand): gallic acid (GA), catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), epigallocatechin (EGC), catechin gallate (CG), caffeine and Trolox. All solvents were of analytical grade, mixtures of C_8-C_{17} n-alkanes, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium (ABTS) were purchased from Merck (Darmstadt, Germany).

2.3 Identification of volatile compounds

A 50/30 µm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) solid-phase microextraction (SPME) fibre purchased from Supelco (Bellefonte, PA, U.S.A.) was chosen to extract the volatile components from all Assam tea samples. The identification of volatile compounds in Assam tea samples was performed according to our previous research (Pripdeevech *et al.*, 2017). For each extraction, the SPME fibre was preconditioned in the injection port of the Agilent 6890 gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA, USA) at 220°C for 1 hr. Approximately, 100 g of each dried Assam tea sample were placed in a 250 mL headspace vial. The sample bottle was preheated at 60°C for 30 mins. The fibre was then exposed to the sample headspace for 30 mins, prior to thermal desorption of the constituents at 250°C into the split-less injection port of the gas chromatography-mass spectrometry (GC-MS) for 5 mins. SPME was performed in triplicate for each sample. The volatile components of all Thai Assam tea samples were analysed using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies) equipped with an HP model 5973 mass-selective detector. An HP-5MS (5% phenylpolymethylsiloxane) capillary column

(30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies) was used in this study. The oven temperature was set to 60°C and then increased to 220°C at a rate of 3°C/min. The injector and detector temperatures were set to 250°C and 280°C, respectively. Purified helium (99.9%) was used as the carrier gas at a flow rate of 1 mL/min. Electron ionization (EI) mass spectra were collected at 70 eV ionization voltages over the range of m/z 29 to 300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set to 230°C and 150°C, respectively. Identification of volatile components was performed by comparison of their Kovát retention indices, relative to C_8-C_{17} n-alkanes, and comparison of the mass spectra of individual components with reference mass spectra in the Wiley7N, NIST05 databases and Adams (2017). The relative contents of volatile compounds were calculated as peak areas to the peak area percentage

2.4 Determination of total phenolic contents

Total phenolic content of Assam tea infusions obtained from different cultivars was determined using the Folin-Ciocalteu reagent according to the method of Pripdeevech and Machan (2011) using gallic acid as standard. The tea solution (0.2 mL) was mixed with 1.0 mL of Folin-Ciocalteu reagent, 1.0 mL of an aqueous solution of 7% Na_2CO_3 and 5.0 mL of distilled water, respectively. Then, the mixture was vortexed vigorously. The reaction mixtures were allowed to stand for 30 min before absorbance at 765 nm was measured. The same procedure was also applied to the standard solutions of gallic acid. The calibration equation for gallic acid was y = 0.00512x-0.00404 (R^2 = 0.999) where x and y are the absorbance and the concentration of gallic acid in mg/mL, respectively. The total phenolic contents of all tea infusions are expressed as mg gallic acid (GA)/g dried weight (dw). The experiment was carried out in triplicate and the results are the mean values.

2.5 Catechins and caffeine analysis

Catechins and caffeine of Assam tea infusions obtained from different cultivars were determined according to the method of Theppakorn (2012). Each Assam tea infusion was prepared by adding 200 mL of boiling distilled water to 2 g of ground tea sample. Stirring using a magnetic stirrer was employed during the brewing process. After 10 mins, the tea infusion was filtered under vacuum and cooled to room temperature. Distilled water was added to tea solution until the final volume was 250 mL. Tea solution was further filtered through a 0.22-μm syringe filter membrane. The filtrate was stored at -20°C for further high-performance liquid chromatography (HPLC) analysis. Analysis of catechins and caffeine was performed using of a Waters model 2695 HPLC system equipped with a model 2996 photodiode array detector. A Waters Platinum EPS C_{18} (7.0 mm i.d. × 53 mm) reversed phase column (Milford, MA). Catechins and caffeine were separated by elution with a flow rate of 2.0 mL/min at 30°C using a binary mobile phase of 0.05% trifluoroacetic acid/acetonitrile (87:13 v/v) in reservoir A and acetonitrile in reservoir B for a total chromatographic run time of 10 mins. Detection of catechins and caffeine was accomplished with a UV detection wavelength of 210 nm. Calibrations plots for quantification were constructed from the injection of authentic standards of catechins and caffeine. Individual catechins and caffeine of each sample were reported as mg compound/g dw. The experiment was carried out in triplicate and the results are the mean values.

2.6 Antioxidant activity

Each Assam tea infusion was prepared by adding 200 mL of boiling distilled water to 2 g of ground tea sample. Stirring using a magnetic stirrer was employed during the brewing process. After 10 mins, the tea infusion was filtered under vacuum and cooled to room temperature. The antioxidant capacity by DPPH assay was evaluated according to the modified method (Insawang *et al.*, 2019). The tea infusion and Trolox were diluted in methanol. A 0.05 mL of various infusions and Trolox was mixed with 1.95 mL of 0.2 mol/L DPPH solution. The mixture was shaken vigorously and kept in the dark at 27°C for 30 mins. The absorbance of the mixture was determined at 517 nm using a spectrophotometer. Methanol was used as a blank solution. The antioxidant activity by ABTS assay was also determined according to the modified method (Insawang *et al.*, 2019). The ABTS radical cation was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and kept in the dark at 27°C. For each concentration, 50 mL of the tea infusion was mixed with 150 mL of ABTS solution before shaking vigorously and kept in the dark at 27°C for 5 mins. The absorbance of the solution was determined at 734 nm using a spectrophotometer. The antioxidant activity of Assam tea cultivars was reported as μmol Trolox/g dw from both assays. Each sample was tested for antioxidant activity in triplicate.

3. Results and discussion

Identified volatile compounds in different tea cultivars analysed by GC-MS and their amounts are summarized in Table 2. Forty-nine volatile compounds were identified. All similar cultivars contained a similar number of volatile components. Monoterpene compounds such as hotrienol, cis and trans-linalool

oxide, linalool, limonene and furfural were found to be the major components among these samples. Assam tea samples obtained from Chiang Rai cultivars present high content of hotrienol, allo-ocimene, Z-β-ocimene, and E-β -ocimene, δ-elemene, and γ-muurolene. Some volatile compounds, jasmone, Z-jasmonyl acetate, and Z-methyl jasmonate were present in Assam tea cultivar obtained from high altitudes such as CM2 and CM4 whilst they were lower in the different cultivar amongst Assam tea samples. It was found that volatile components have been changed in term of quality and quantity according to the tea cultivar (Pripdeevech and Machan, 2011).

The phenolic contents of 22 Assam tea cultivars are shown in Figure 1. Among all cultivars analysed, NN2 had the highest phenolic content (248.45±0.12 mg GA/g dw). CR4 cultivar had the lowest phenolic content (113.06±0.13 mg GA/g dw). In this study, there was a 2.2-fold difference in phenolic content between the highest and lowest-ranked Assam tea cultivars. The phenolic compounds being a large group of secondary metabolites in natural products are evaluated to be responsible for the health benefits in cardiovascular protection and anticancer effects associating with their strong antioxidant activities (Zhou *et al.*, 2016).

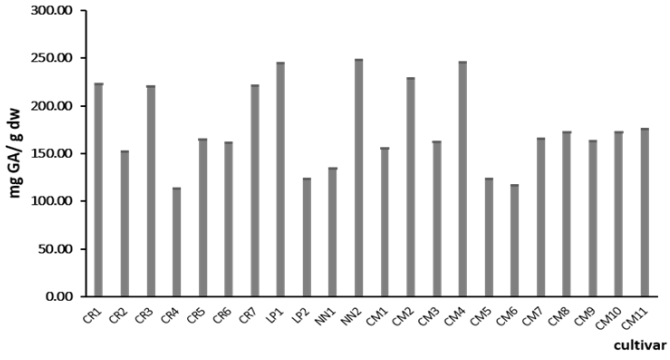

Figure 1. Total phenolic content of 22 Assam tea cultivars

The contents of represented catechins and caffeine were analysed by HPLC and the results are shown in Figure 2. All cultivars provided caffeine content ranging between 0.92-3.41 mg/g dw. Among all the tea cultivars analysed, CM5 and LP1 cultivars showed the lowest and highest caffeine content, respectively. Most tea cultivars had higher caffeine with more than 2.00 mg/g dw except for CM5, CM6, and CM7 cultivar which was lower than 1.0 mg/g dw. In addition, CM4 had the highest total catechins content (355.59±0.14 mg/g dw). CM10 had the lowest total catechins content (170.03±0.78 mg/g dw). In evaluating tea cultivars for phenolic and catechin contents, a wide range has existed among these cultivars. The difference in content was mainly attributed to the different cultivars of Assam tea samples grown in the same conditions used in this study as reported by Fujimura et al. (2011). The amount of catechins in tea cultivar may result mainly in flavour and colour of tea and may be useful for the classification of tea cultivars and tea-making suitability (Wink, 2003). Catechins (EGCG, GC, EGC, and ECG) have been evaluated as the major phenols in almost all kinds of teas (Tong *et al.*, 2019). EGCG has been reported as the richest catechins in tea and shown the strongest antioxidant activity in catechins, which is stronger than vitamins C and E (Zhao *et al.*, 2014). It could possess several bioactivities such as anticancer activity by inhibiting cancer stem cells and modulating molecular actions associated with cancer cell proliferation, apoptosis, and immunity (Gan *et al.*, 2018). Among all the Assam tea cultivars, CR7, CM4, CM2, and NN2 cultivars had high EGCG content (more than 134.00 mg/g dw). Other cultivars such as CR1, CR3, and LP1 showed moderate EGCG content ranging 113.23±0.29 to 123.67±1.24 mg/g dw. Other cultivars had low EGCG content (lower than 100.00 mg/g dw). In this study, there was an approximately 2.3-fold difference in EGCG content between the highest and lowest-ranked cultivars. GC is another major catechin detected in all tested Assam tea cultivars. GC content ranged from 45.78 (CM10) to 128.77 (LP1) mg/g dw. There were almost 2.8-fold differences in GC contents between CM10 and LP1. Wolfram *et al*. (2006) reported that a bitter and astringent taste in tea was attributed to GC content. Therefore, cultivars of Assam tea samples from CR7, CR3, CM4, CM2, NN2, and LP1 had a similar bitter taste due to the high content of GC

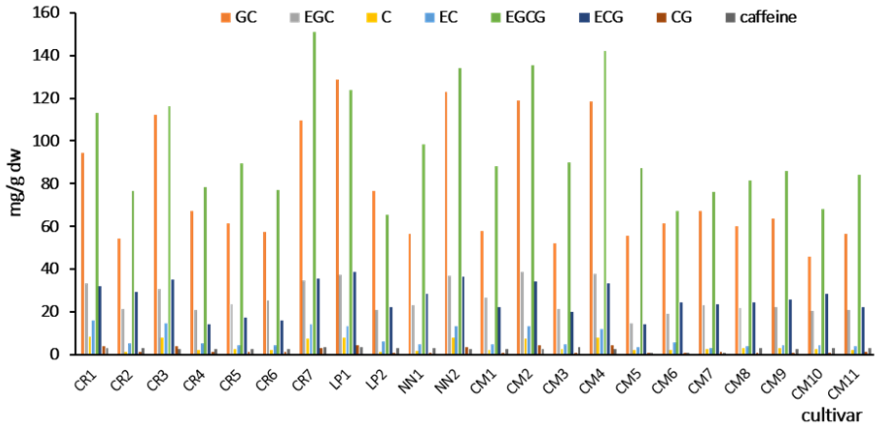

Figure 2. Catechins and caffeine content of 22 Assam tea cultivars

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Table 2. Identified volatile compounds of 22 Assam tea cultivars

No.	Compound	LRI	Average relative peak area (%)																				
			CR1	CR2	CR3	CR4	CR5	CR6	CR7	LP1	LP2	NN1	NN2	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8	CM9	CM10	CM11
1	Furfural	827	3.34	3.25	2.56	3.58	3.25	3	2.96	5.86	6.25	3.58	2.58	3.25	0.25	3.21	0.35	3.63	3.35	3.66	3.05	3.08	3.69	3.98
2	trans-Isolimonene	984	7.88	7	7.5	6.96	6.98	6.38	8.5	4.51	4.35	0.58	0.98	4.52	0.56	4.56	0.58	4.85	4.98	5.06	5.11	5.23	5.69	5.88
3	δ-3-Carene	1011	3.05	2.56	3.25	3	3.15	3.69	3.45	3.65	3.68	0.69	0.85	3.25	0.78	2.25	0.78	3.63	3.33	3.05	3.44	2.96	2.98	2.78
4	α-Terpinene	1017	1.03	0.96	0.85	0.98	0.63	0.98	1.05	1.25	1.36	0.77	0.87	3.14	0.14	3.05	0.25	3.09	3.14	3.66	3.58	3.55	3.2	3.07
5	Limonene	1029	4.56	4.8	4.96	4.87	5.02	5.11	5.23	0.58	0.66	25.87	20.25	15.96	6.54	18.96	7.52	17.52	18.36	18.05	19.05	17.69	18.52	19.63
6	1,8-Cineole	1031	2.66	2.31	2.21	2.36	3.15	3	3.56	0.28	0.28	6.35	7.85	0.15	0.23	0.09	0.36	0.17	0.18	0.08	0.09	0.11	0.17	0.12
7	*Z-β-Ocimene*	1037	2.18	2	2.15	2	2.5	2.31	2.21	0.56	0.85	0.11	0.21	0.52	0.36	0.62	0.45	0.71	0.63	0.66	0.68	0.61	0.63	0.6
8	*E-β-Ocimene*	1050	2.45	2.25	2.36	2.58	2.36	2.58	2.22	0.89	0.89	0.05	0.15	0.68	0.35	0.6	0.11	0.6	0.65	0.63	0.62	0.61	0.59	0.65
9	γ-Terpinene	1059	1.37	1.25	1.36	1.58	1.36	1.45	1.58	0.99	0.85		0.05	0.75	0.47	0.7	0.15	0.78	0.84	0.82	0.74	0.82	0.71	0.7
10	cis-Linalool oxide	1072	4.95	6.25	5.36	4.99	4.98	5.12	5.18	6.33	5.96	0.58	0.88	10.25	3.58	11.23	3.21	10.98	11.56	12.25	12.03	12.74	11.92	12.56
11	trans-Linalool oxide	1085	3.83	1.05	4.11	3.95	3.88	3.98	4.15	5.26	5.36	0.68	0.47	2.54	3.69	2.63	3.32	2.58	2.75	2.6	2.8	2.42	2.2	2.45
12	Terpinolene	1088	0.08	0.08		0.05		0.04		0.69	0.78	0.47	0.89	0.36	2.14	0.3	2.05	0.42	0.45	0.41	0.36	0.35	0.36	0.32
13	Linalool	1096	2.56	2	2.36	2.04	2.11	2.01	2.08	15.56	16.85	20.35	15.69	5.89	12.54	5.96	18.15	6.32	6.52	5.87	5.29	5.64	5.87	6.05
14	Hotrienol	1108	20.85	22.85	23.56	22.55	23.58	22.47	20.58	18.56	19.65	12.58	18.54	15.63	15.58	18.52	0.22	18	17.79	18.23	19.25	17.44	18.89	19.06
15	endo-Fenchol	1116	0.05		0.05	0.06			0.08	0.66	0.52	0.05	0.11	2.36	0.25	2.3	0.05	2.47	2.52	2.39	2.85	2.74	2.56	2.56
16	allo-Ocimene	1128	7.58	7.85	7.12	7.05	7.15	7.36	7.28	0.58	0.58	0.11	0.18	2.56	0.45	2.23	0.41	2.45	2.61	2.54	2.63	2.54	2.55	2.63
17	Isoborneol	1161	3.46	3.58	2.98	3.98	3.78	3.56	3.66	0.14	0.21		0.05	0.14	0.78	0.13	0.7	0.15	0.12	0.11	0.09	0.11	0.09	0.05
18	a-Terpineol	1186	0.71	0.52	0.65	0.86	0.49	0.57	0.69	0.18	0.23	0.08	0.05	0.18	0.69	0.11	0.54	0.18	0.17	0.09	0.11	0.15	0.14	0.14
19	Linalool formate	1216	0.05	0.05	0.09		0.09	0.11		0.95	0.98	0.58	0.98	3.65	0.68	3.65	0.62	3.25	3.35	3.65	3.45	3.85	3.55	3.64
20	Nerol	1227	0.08	0.05	0.09	0.11		0.11		0.56	0.62	0.08	0.05	0.05	0.55	0.09	0.5	0.09	0.11	0.05		0.05	0.05	0.09
21	*E-Ocimene*	1238	0.09	0.08	0.11	0.15	0.18	0.09	0.18	0.44	0.48	0.47	0.78	0.14	0.21	0.1	0.2	0.15	0.15	0.26	0.22	0.23	0.25	0.22
22	Isobornyl formate	1239	0.84	0.84	0.75	0.85	0.86	0.85	0.85	0.32	0.36	0.28	0.89	0.19	0.15	0.1	0.14	0.11	0.15	0.16	0.17	0.1	0.09	0.18
23	Linalool acetate	1257	0.08	0.09	0.14	0.12	0.09	0.05	0.17	0.22	0.36	0.29	0.65	0.08	5.45	0.05	4.52	0.05	0.05		0.05	0.1	0.09	0.11
24	Dihydro-linalool acetate	1275	0.71	0.89	0.88	0.87	0.82	0.77	0.79	0.11	0.18	0.32	0.38	0.18	2.85	0.18	2.05	0.11	0.09	0.14	0.09	0.14	0.11	0.15
25	Indole	1294	0.05	0.08		0.05	0.08			0.36	0.38	0.74	0.85	2.55	0.56	2.56	0.41	2.54	2.33	2.36	2.58	2.64	2.57	2.22
26	δ-Elemene	1325	2.51	2.5	2.25	2.36	2.87	2.88	2.56	0.54	0.55	0.11	0.63	0.25	0.65	0.25	0.21	0.22	0.21	0.26	0.23	0.24	0.24	0.22
27	α-Cubebene	1338			0.05	0.05	0.09			0.22	0.29	0.09	0.11	0.33	0.38	0.3	0.29	0.29	0.31	0.31	0.33	0.35	0.33	0.31
28	Calacorene	1342	1.02	0.98	0.89	1.01	1.08	1.08	1.11	1.08	1.15	0.05	0.09	0.14	0.98	0.1	0.87	0.11	0.09	0.11	0.14	0.15	0.11	0.09
29	α-Ionene	1348			0.05			0.05		0.99	0.99		0.05	0.18	0.17	0.17	0.17	0.11	0.11	0.09	0.15	0.15	0.14	0.18
30	α-Longipinene	1352								0.08	0.07			0.05	0.19	0.05	0.11		0.05					

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Table 2 (Cont.). Identified volatile compounds of 22 Assam tea cultivars

No.	Compound	LRI	Average relative peak area (%)																				
			CR1	CR2	CR3	CR4	CR5	CR6	CR7	LP1	LP2	NN1	NN2	CM1	CM2	CM3	CM4	CM5	CM6	CM7	CM8	CM9	CM10	CM11
31	α-Copaene	1370	1.58	1.25	1.36	1.55	1.56	1.58	1.62	0.08	0.09	0.14	0.18	0.09	0.56	0.11	0.52	0.05	0.05		0.05	0.05		
32	*Z-Jasmone*	1392	0.62	0.65	0.65	0.69	0.63	0.59	0.58	8.56	8.78	0.52	0.65	0.15	20.56	0.11	22.41	0.15	0.12	0.13	0.14	0.11	0.18	0.14
33	α-Gurjunene	1409	0.64	0.59	0.65	0.63	0.66	0.58	0.64	0.22	0.32	0.78	0.89	0.52	0.54	0.52	0.5	0.56	0.45	0.47	0.59	0.52	0.54	0.55
34	2-epi-β-Funebrene	1412	0.62	0.58	0.58	0.56	0.61	0.63	0.66	0.32	0.48	0.18	0.22	0.26	0.52	0.12	0.47	0.22	0.25	0.24	0.14	0.26	0.24	0.2
35	β-Cedrene	1420	0.85	0.85	0.82	0.88	0.88	0.8	0.91	0.33	0.41	0.19	0.87	0.78	0.28	0.6	0.21	0.52	0.63	0.53	0.52	0.5	0.49	0.44
36	Neryl acetone	1436	0.58	0.58	0.52	0.49	0.44	0.47	0.56	0.09	0.05	0.36	0.36	0.47	0.37	0.4	0.31	0.42	0.41	0.33	0.45	0.47	0.44	0.4
37	γ-Elemene	1438			0.05	0.05	0.05		0.08	0.15	0.11	0.54	0.78	0.56	0.16	0.5	0.11	0.44	0.39	0.44	0.45	0.42	0.5	0.48
38	*Z-Jasmonyl acetate*	1455		0.05	0.09				0.05	4.58	3.56	0.25	0.35	0.25	15.26	0.21	14.58	0.2	0.11	0.25	0.23	0.24	0.24	0.22
39	9-epi-*E-Caryophyllene*	1466	0.95	0.98	0.99	0.89	0.82	0.99	0.98	0.52	0.57	0.88	0.94	1.89	0.05	1.8	0.1	1.74	1.7	1.72	1.45	1.65	1.52	1.48
40	γ-Muurolene	1479	2.18	2.05	2.11	2.03	2.01	2.08	2.06	0.36	0.45	0.74	0.85	1.89	0.05	1.4		1.42	0.13	1.25	1.26	1.35	1.21	1.52
41	Germacrene D	1485	1.89	1.8	1.59	1.63	1.98	1.47	1.58	0.47	0.58	7.84	8.63	1.63	0.11	1.42	0.09	1.4	1.36	1.35	1.33	1.36	1.4	1.39
42	*E-β-Ionone*	1489	2.14	2.3	2.22	2.36	2.42	2.33	2.42	0.58	0.63	0.28	0.56	1.88		1.5	0.05	1.02	1.26	1.03	1.05	1.12	1.1	1.05
43	α-Muurolene	1500	0.61	0.58	0.55	0.48	0.52	0.56	0.51	0.11	0.05	0.63	0.53	1.45		0.52		0.26	0.25	0.36	0.45	0.48	0.56	0.5
44	a-(E,E)-Farnesene	1505	0.53	0.5	0.52	0.55	0.52	0.54	0.44	0.1	0.05	0.44	0.64	0.25		0.2		0.25	0.2	0.2	0.1	0.1	0.11	0.05
45	Germacrene A	1509	0.56	0.45	0.48	0.56	0.52	0.35	0.44	0.89	0.96	2.55	2.14	1.09		0.05	0.05	0.05	0.1	0.1	0.11		0.05	
46	Cubebol	1515	2.58	2.8	2.87	2.56	2.48	2.63	2.89	0.88	0.98	0.58	0.68	0.25		0.02		0.25	0.1	0.25	0.05			
47	trans-Calamenene	1521	0.62	0.78	0.77	0.75	0.88	0.89	0.77	0.59	0.63	3.68	3.02	0.86		0.98		0.95	0.98	0.99	0.09	0.98	0.85	
48	E-Nerolidol	1561	1.14	1.23	1.35	1.41	1.32	1.32	1.55	1.56	1.67	0.66	0.52	0.22	0.14	0.1	0.05	0.11	0.05	0.05	0.05	0.05		
49	Z-Methyl jasmonate	1648	0.56	0.85	0.86	0.82	0.8	0.74	0.79	3.59	2.87			0.58	10.58	0.25	9.25	0.15	0.26	0.14	0.05	0.05	0.09	
	Total of compounds		96.67	94.99	97.72	97.9	99.63	98.15	99.65	96.38	98.96	97.15	98.92	94.99	96.38	95.86	97.99	95.72	95.75	97.38	97.74	96.5	97.81	99.06

detected. Moreover, EGC and ECG contents were also detected as the major catechins in all cultivars ranging from 14.34-38.87 mg/g dw and 14.23-38.45 mg/g dw, respectively. Among all the tested Assam tea cultivars, the phenolic and catechin contents of different Assam tea cultivars depended largely on the varietal difference (Zeng *et al.*, 2016). Moreover, a previous study also demonstrated that the various contents of major catechin compounds including EGCG, GC, ECG and EGC in tea infusions were also related to the brewing time (Koch *et al.*, 2017). Therefore, the optimization of brewing time may be further determined.

Due to the complexity of phytochemical compounds in tea, the antioxidant activities of tea were also evaluated. For this study, two assays, DPPH and ABTS were employed to determine comprehensively the antioxidant activities of tea infusions. The antioxidant activities of 22 Assam tea cultivars determined by both assays are expressed as µmol Trolox/g dw as shown in Figure 3. The antioxidant activity by DPPH assay (Figure 3A) ranged from 1234.45±0.17 µmol Trolox/g dw (CR2) to 2728.46±0.24 µmol Trolox/g dw (LP1). There was a 2.2-fold difference in DPPH values between the highest and lowest-ranked cultivars, CR2 and LP1. The ABTS values of 22 Assam tea cultivars are depicted in Figure 3B which was also expressed as µmol Trolox/g dw. Variation of ABTS values ranged from 1448.98±1.31 µmol Trolox/g dw (NN1) to 2864.97±1.12 (NN2) µmol Trolox/g dw. There was a 2.0-fold difference in ABTS value between the lowest and highest ranked cultivars, NN1 and NN2. Similar contents of antioxidant activity of 22 Assam tea cultivars were detected in both assays although some minor differences of antioxidant activity using DPPH and ABTS assays were detected. The values obtained from DPPH and ABTS of 22 tea infusions indicated that phenolic compounds were responsible for the strong antioxidant activities regarding reducing and free radicals scavenging abilities. However, it should be noted that the antioxidant activity of 22 Assam tea cultivars may be correlated to the presence of non-phenolic compounds such as polysaccharides. Sun *et al.* (2018). reported that four polysaccharides detected in green tea, with a molecular weight of 10.88, 8.16, 4.82, and 2.31 kDa possessed hydroxyl and ABTS radical scavenging activity.

From our results above, it can be noted that all Assam tea cultivars were evaluated to be the important tea having a good quality of antioxidant activity with an individual volatile profile. High antioxidant activity, total phenolic content and catechin compounds among these cultivars could be related to the low oxidation reaction of young tea leaves and bud due to inactivated enzymes during the sample preparation process (Senanayake, 2013). This observation is in agreement with the study of Zeng *et al.* (2016). The results suggested that tea cultivars had different antioxidant activity due to different phytochemicals. In addition, tea cultivars obtained from high altitudes above 1000 m such as CR1, CR3, CR7, LP1, NN2, CM2, and CM4 showed a higher amount of major catechin compounds than those obtained from other cultivars. Altitude maybe seemed to strongly outweigh their contribution due to the same tea production process.

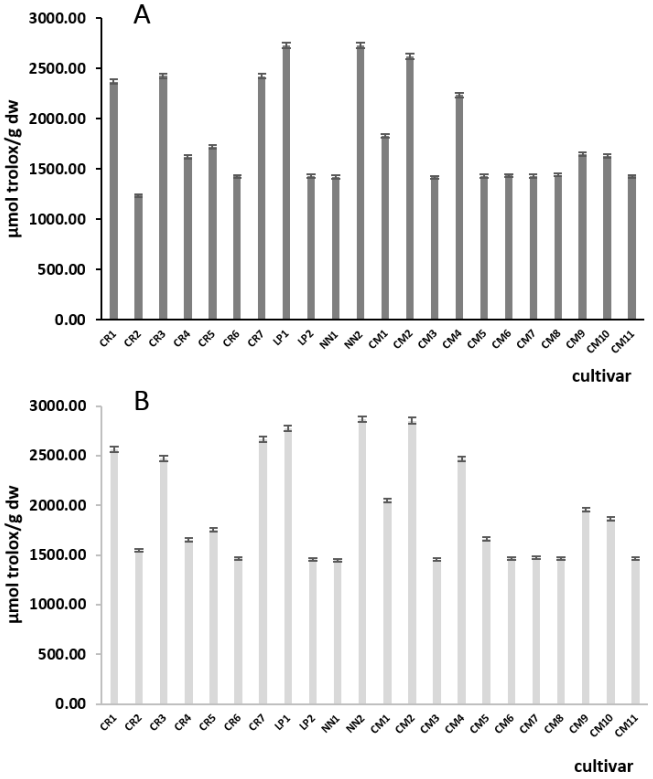

Figure 3. Antioxidant activity of 22 Assam tea cultivars by DPPH (A) and ABTS (B) assay

4. Conclusion

The present study comparatively determined the volatile profiles, total phenolic content, catechins, and antioxidant activity of Thai Assam tea cultivars grown in the same condition for the first time. The results suggested that 22 Assam tea infusions possessed specific volatile compounds characteristic, different antioxidant activities, phenolic content and catechin compounds varying in specific cultivars. The total antioxidant activities of Assam teas may be correlated with the major phenolic compounds among these cultivars such as EGCG, GC, ECG, and EGC. Tea cultivars obtained from high altitude show higher antioxidant activity, total phenolic and catechins content compared to other cultivars. Therefore, further research is needed to investigate the synergistic and individual effects of phytochemical compounds responsible for the antioxidant activity of Thai Assam tea. Knowledge of

specific differences in the volatile profiles and antioxidant activities among Thai Assam tea cultivars is important data that may be used for breeding Assam tea cultivars in selecting for tea production in Thailand with various health benefits.

Conflict of interest

The authors declare no conflict of interest.

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References

Adams, R.P. (2017). Identification of essential oil components by gas chromatography/mass spectrometry. Illinois, USA: Allured publishing Corporation: Carol Stream.

Afzal, M., Safer, A.M. and Menon, M. (2015). Green tea polyphenols and their potential role in health and disease. *Inflammopharmacology*, 23, 151-161. https://doi.org/.org/10.1007/s10787-015-0236-1

Almajano, M.P., Carbó, R., Jiménez, J.A.L. and Gordon, M.H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, 108(1), 55-63. https://doi.org/10.1016/j.foodchem.2007.10.040

Astill, C., Birch, M.R., Dacombe, C., Humphrey, P.G. and Martin, P.T. (2001). *Journal of Agricultural and Food Chemistry*, 49, 5340-5347. https://doi.org/10.1021/jf010759

Fujimura, Y., Kurihara, K., Ida, M., Kosaka, R., Miura, D., Wariishi, H., Maeda-Yamamoto, M., Nesumi, A., Saito, T., Kanda, T., Yamada, K. and Tachibana, H. (2011). Metabolomics-driven nutraceutical evaluation of diverse green tea cultivars. *PLoS One*, 6, e23426. https://doi.org/10.1371/journal.pone.0023426

Gan, R.Y., Li, H.B., Sui, Z.Q. and Corke, H. (2018). Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): An updated review. *Critical Reviews in Food Science and Nutrition*, 58(6), 924-941. https://doi.org/10.1080/10408398.2016.1231168

Insawang, S., Pripdeevech, P., Tanapichatsakul, C., Khruengsai, S., Monggoot, S., Nakham, T., Artrod, A., D'Souza, P.E. and Panuwet, P. (2019). Essential oil compositions and antibacterial and antioxidant activities of five *Lavandula stoechas* cultivars grown in Thailand. *Chemistry and Biodiversity*, 16(10), e1900371. https://doi.org/10.1002/cbdv.201900371

Koch, W., Kukula-Koch, W. and Glowniak, K. (2017). Catechin composition and antioxidant activity of black teas in relation to brewing time. *Journal of AOAC International*, 100(6), 1694-1699. https://doi.org/10.5740/jaoacint.17-0235

Kumazawa, K. and Masuda, H. (2002). Identification of potent odorants in different green tea varieties using flavor dilution technique. *Journal of Agricultural and Food Chemistry*, 50, 5660-5663. https://doi.org/10.1021/jf020498j

Li, F.-J., Ji, H.-F. and Shen, L. (2012). A meta-analysis of tea drinking and risk of Parkinson's disease. *The Scientific World Journal*, 2012, 923464. https://doi.org/10.1100/2012/923464

Phromrukachat, S., Tiengburanatum, N. and Meechui, J. (2010). Assessment of active ingredients in pickled tea. *Asian Journal of Food and Agro-Industry*, 3(3), 312-318.

Pripdeevech, P. and Machan, T. (2011). Fingerprint of volatile flavour constituents and antioxidant activities of teas from Thailand. *Food Chemistry*, 125(2), 797-802. https://doi.org/10.1016/j.foodchem.2010.09.074

Pripdeevech, P., Rothwell, J., D'Souza, P.E. and Panuwet, P. (2017). Differentiation of volatile profiles of Thai Oolong tea No. 12 provenances by SPME-GC-MS combined with principal component analysis. *International Journal of Food Properties*, 20(Sup. 3), S2450-S2462. https://doi.org/10.1080/10942912.2017.1374288

Reichart, P.A. and Philipsen, H.P. (2005). Betel and Miang: Vanishing Thai Habits. Bangkok, Thailand: White Lotus Books.

Senanayake, S. (2013). Green tea extract: Chemistry, antioxidant properties and food applications-A review. *Journal of Functional Foods*, 5(4), 1529-1541. https://doi.org/10.1016/j.jff.2013.08.011

Sun, X.Y., Wang, J.M., Ouyang, J.M. and Kuang, L. (2018). Antioxidant activities and repair effects on oxidatively damaged hk-2 cells of tea polysaccharides with different molecular weights. *Oxidative Medicine and Cellular Longevity*, 2018, 5297539. https://doi.org/10.1155/2018/5297539

Theppakorn, T. (2012). Tea (*Camellia sinensis* L.): Manufacturing and chemical compositions from fermentation. *Burapha Science Journal*, 17, 145-152. [In Thai].

Theppakorn, T., Luthfiyyah, A. and Ploysri, K. (2014). Comparison of the composition and antioxidant capacities of green teas produced from the Assam and the Chinese varieties cultivated in Thailand. *Journal of Microbiology, Biotechnology and Food*

Sciences, 3(5), 364-370.

Theppakorn, T., Yamee, K. and Ekkapan, A. (2012). Green tea catechins and storage stability. *KKU Science Journal*, 40(1), 1225-1235. [In Thai].

Tong, T., Liu, Y.J., Kang, J., Zhang, C.M. and Kang, S.G. (2019). Antioxidant activity and main chemical components of a novel fermented tea. *Molecules*, 24 (16), 2917. https://doi.org/10.3390/ molecules24162917

Wink, M. (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, 64(1), 3-19. https:// doi.org/10.1016/S0031-9422(03)00300-5

Wolfram, S., Wang, Y. and Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Molecular Nutrition Food Research*, 50(2), 176-187. https://doi.org/10.1002/mnfr.200500102

Zeng, L., Luo, L., Li, H. and Liu, R. (2016). Phytochemical profiles and antioxidant activity of 27 cultivars of tea. *International Journal of Food Sciences and Nutrition*, 68(5), 525-537. https:// doi.org/10.1080/09637486.2016.1263834

Zhao, X., Wang, Q., Li, G., Chen, F., Qian, Y. and Wang, R. (2014). In vivo antioxidant, anti-mutagenic, anti-cancer and antiangiogenic effects of Chinese bowl tea. *Journal of Functional Foods*, 7, 590-598. https://doi.org/10.1016/j.jff.2013.12.026

