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Catechins and Caffeine Contents of Green Tea Commercialized in Thailand

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Abstract:

A validated reversed-phase HPLC methodhas been employed for the determination of five individual catechins; (-) epigallocatechin gallate (EGCG), (-) epigallocatechin (EGC), (-) epicatechin gallate (ECG), (-) epicatechin (EC), and (+) catechin (C), and caffeine in green tea simultaneously.The separation was performed on a C_{18}-bonded silica column (4.6 x 250 mm, 5µm) using an isocratic mobile phase comprising of 0.1% acetic acid and methanol (70:30, v/v) with a UV detection at 230 nm. The method validation showed good results for specificity, linearity, precision, accuracy, limit of detection and limit of quantitation. The content of 5 catechins and caffeine in commonly consume green tea brands in Thailand were determined and compared. The resulting data revealed extensive variability in catechins and caffeine amounts. The quantities of EGCG ranged from 5.19 to 58.21, EGC from 2.80 to 52.48, EC from 0.74 to 11.58, ECG from 1.01 to 16.45 and C from 0.09 to 6.10 mg/g of dry tea. Caffeine contents were between 5.81-27.62mg/g of dry tea. EGCG was in the highest concentration in almost tea infusions excepting Japanese green tea, while C was the lowest, excepting Chinese green tea.

Key words: Green tea, Catechins, Caffeine, HPLC.

Introduction:

Tea (*Camellia sinensis* (L.) Kuntze)) is one of the most widely consumed beverage in Thailand with increasing attention to the consumption of green tea because of its variety biological activities known to improve health conditions[1-7]. Two main groups which play an important role on their interesting activities in green tea such as antioxidant, antibacterial, hypocholesterolemic and antitumor actions are catechins and xanthines. The major active catechins are (-) epigallocatechingallate (EGCG), (-) epigallocatechin (EGC), (-) epicatechin gallate (ECG), (-) epicatechin (EC), and (+) catechin (C) whereas the major active xanthines is caffeine. Recently, green tea is being used in topical skin care preparations and cosmetics for protecting collagen from being broken down. Furthermore, it has been widly applied in many kind of foods, bakeries and desserts due toits strong scent[8].

Tea is native to China, India, Laos, Thailand, Vietnam and Myanmar[8]. In Thailand, tea is cultivated in the northern part and in manufacturing, green teais produced from 2 varieties, *C. sinensis* var. *assamica* and *C. sinensis* var. *sinensis*. Moreover, green tea products have also been imported from Japan and China. Two types of Japanese green tea; *sencha* and *bancha*, are commonly consumed while two types of Chinese green tea; *biluochun* and *longjing*, are accessible. Individual catechins hasvarious anti-oxidative ability as it is observed that the antioxidant activity is higher in green tea that contains higher levels of EGCG and EGC[9]. Caffeine is known for its stimulatory effects as well. There is an evidence of health risks for consuming high amount of caffeine such as tachycardia, insomnia, nervousness, diarrhea and diuresis[10]. Analysis of bioactive compounds is subsequently needed to facilitate the production of good quality green tea.

Extensive literature survey reveals that various analytical methods have been reported for the determination of catechins and caffeine in tea[11-18]. However, a few data on the quantities of catechins and caffeine of Thai green tea has been reported[19-21]. Therefore, the levels of 5 individual catechins (EGCG, EGC, ECG, EC and C) and caffeine in Thai green tea infusion were established. Additionally, Japanese green tea and Chinese green tea commercially available in Thailand were determined and compared. In order to obtain accurate data, this study will also focus on a reversed-phase HPLC analytical determination. The method is verified and validated with respected to linearity, accuracy, precision, limit of detection and limit of quantitation.

Materials and methods:

Materials and reagents

Thirty green tea brands manufactured in Thailand, Japan and China were purchased from local markets or supermarkets in Bangkok, Thailand. Fifteen green tea brands manufactured in Thailand were divided to 3 groups; firstly, 7 samples produced from *C. sinensis* var. *assamica* (TH1-TH7), secondly, 5 samples produced from *C. sinensis* var. *sinensis* (TH8-TH12) and finally, 3 imported Japanese "*sencha*" raw materials and packed in Thailand (TH13-TH15). Seven "*sencha*" (JP1-JP7) and three "*bancha*"

(JP8-JP10) samples manufactured in Japan were employed, whereas two "*biluochun*" (CH1-CH2) and three "*longjing*" (CH3-CH5) Chinese samples were utilized.

Five catechin standards; (+) catechin (C), (-) epigallocatechin gallate (EGCG), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC), and(-) epicatechin (EC), and caffeine were purchased from Sigma (St. Louis, MO, USA) with purities greater than 98 %. HPLC-grade methanol was supplied by Merck (Darmstadt, Germany), other chemicals used were of analytical grade. HPLC grade water (18 megaohm), prepared using a Nanapure purification system, was used to prepare all solutions.

Instrumentation and chromatographic conditions

SP thermo separation model HPLC system consisted of a Spectra SYSTEM Constametric 4100 solvent delivery system with a membrane degasser, a Spectra series autosampler AS 3000, a Spectro Monitor 4100 detector and the PC 1000 data analysis software. The analytical column was a Symmetry-C18 reversed-phase, 250 mm x 4.6 mm i.d., packed with 5 µm C18 modified silica (Water, USA). A 20 mm x 3.9 mm i.d. guard column packed with 5-µm diameter Symmetry-C18 was utilized.

The chromatographic separation was performed under an isocratic condition. The mobile phase consisted of 0.1% acetic acid and methanol (70:30, v/v). The mobile phase was filtered through Nylon66 membrane filters (47 mm, 0.45 µm) and degassed by an ultrasonic bath. A flow rate of 1.0 ml/min was maintained and a UV detection was set at 230 nm. An aliquot of sample solution (10 µl) was injected onto the analytical column with an auto HPLC injector.

Preparation of standard solutions

Stock solutions of each standard were prepared as aqueous solution with concentration range of 0.8-1 mg/ml. A standard mixture solution containing 5-67 µg/ml of each of the five catechins (EGCG, EGC, EC, ECG and C) and caffeine was prepared in water and used in all method optimization and quantitative analysis.

Preparation of sample solutions

Tea-leaf samples were prepared by pouring 140 mlhot ultra-pure water (80°C) over a 1 g sample and allowed to draw for 3 min with occasional stirring. The leaves were separated from the tea infusion by filtering through a filter paper (Whatman No.1). It was then transferred into a 250 ml flask and adjusted to volume with ultra-pure water. Subsequently, the tea infusions were filtered through a 0.45 µm membrane filter and a 10 µl of tea samples was analyzed directly by HPLC. Each sample was analyzed for individual catechins and caffeine content in six replicate.

Data analysis

Linear regression analysis of the calibration curve was evaluated using data analysis MS excel software (2007) (ANOVA; $P < 0.05$). Differences in the average individual catechin contents of tea samples were tested to be statistically significant at $p < 0.05$.

Results and discussion:

Separations and specificity

The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. Several methanol aqueous-based mobile phase and acetonitrile aqueous-based mobile phase were studied in combination with phosphoric acid and acetic acid. It was found that 0.1% acetic acid: methanol in the proportion of 70:30 was successfully separated five important catechins and caffeine within 15 min. The retention times for EGC, C, EGCG, caffeine, EC and ECG were observed to be 3.7, 4.1, 4.9, 5.6, 6.6 and 10.5 min, respectively. In addition, the retention time of catechin gallate (CG), and other two xanthines, theobromine and theophylline were checked and it was found to be 13.4, 3.1 and 4.4 min, respectively. Their peaks were sufficiently separated from six compounds above and no interferences in the tea sample were encountered. The detection wavelength at 210, 230, 270 and 280 nm have been studied. Detection wavelength at 230 nm was found to give better sensitivity for catechins than at 270 nm and 280 nm and problem of interference from other organic compounds presented in green tea detected at 210 nm could be avoided. Therefore this wavelength was chosen for analysis. Typical chromatograms obtained from a standard mixture solution and a tea sample solution at optimum condition is shown in **Figure 1**. These results indicate a specificity of the proposed method.

System suitability parameters were evaluated. The capacity factor values were found to be within the range of $2.3<k'<8.5$, the resolution between two adjacent peaks was greater than 1.6, tailing factors were < 1.2 and number of theoretical plates was >2840. The results verified that the chromatographic system was suitable for analysis of catechins and caffeine in green tea.

Method validation

Linearity and range

Linearity of the individual catechins (EGCG, EGC, EC, ECG and C) and caffeine were determined by analysis of three replicates of six concentrations (range from 5-67 µg/ml) by least squares regression. The relationship of detector response measured as peak area versus concentration of five catechins and caffeine were investigated. The standard curves for each catechin and caffeine showed good linearity over the selected concentration. The correlation coefficient of calibration curves were greater than 0.997. The slope, intercept and correlation coefficient are listed in **Table 1**.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) were established at a signal-to-noise (S/N) ratio of three and ten respectively. LOD and LOQ were experimentally verified by ten injections. LOD ranged from 0.2-2.1 µg/ml and LOQ ranged from 0.66-6.8 µg/ml (RSD $\leq$ 6.61%, n=10). This suggests that this method is sufficiently sensitive for determining catechins and caffeine in tea samples (**Table 1**).

Table 1. Statistical data for calibration plots, LOD and LOQ.

	Linear range (µg/ml)	Slope ($\cdot 10^4$)	Intercept ($\cdot 10^4$)	r^2	LOD (µg/ml)	LOQ (µg/ml)
EGC	10.20 - 61.20	1.6	-0.00	0.9991	0.40	1.54
C	11.05 - 66.30	2.0	-2.9	0.9994	0.20	0.66
EGCG	9.00 - 54.00	1.5	-5.2	0.9981	0.72	2.20
EC	10.00 - 60.00	2.0	-2.5	0.9998	0.30	1.00
ECG	10.30 - 61.80	1.5	-8.5	0.9980	2.10	6.80
Caffeine	5.17 - 30.99	1.2	-0.9	0.9992	0.50	1.55

Accuracy

The accuracy of the method was determined by investigating the recovery studies of five catechins and caffeine. A series of known amount of each catechin and caffeine standard in the range from 5-67 µg/ml was added to aliquots of green tea samples, mixed, filtered and diluted to yield 4 concentrations of each catechin and caffeine. Assays were performed in three replicates at each concentration on three sample brands. The percentage recovery of added catechins and caffeine was calculated by comparing the peak area of the test samples with that of the standard solutions. The accuracy data is presented in **Table 2**. The mean recoveries of five catechins; EGC, C, EGCG, EC and ECG ranged from 103.81-105.84, 97.26-108.60, 106.06-107.94, 103.95-110.70 and 101.89-111.94 %, respectively. The recovery of added caffeine ranged from 104.17-105.04 %. The RSD of recovery studied was between 2.99 to 8.16%. The result is found to be satisfactory for intended purpose and is adequate for routine analysis.

Table 2. Recoveries of catechins and caffeine from three tea samples.

	Sample 1		**Sample 2**		**Sample 3**	
	*Recovery (%)±SD	%RSD	*Recovery (%)±SD	%RSD	*Recovery (%)±SD	%RSD
EGC	105.84±5.48	5.18	103.81±5.45	5.25	104.95±3.31	3.15
C	108.60±6.84	6.29	104.30±7.90	7.58	97.26±7.93	8.16
EGCG	107.94±7.98	7.39	106.59±5.05	4.74	106.06±3.17	2.99
EC	110.70±8.78	7.93	103.95±7.17	6.89	109.49±4.47	4.08
ECG	105.45±4.05	3.84	111.94±5.74	5.14	101.89±3.23	3.17
caffeine	104.57±5.75	5.50	104.17±6.04	5.80	105.04±5.09	4.84

*Mean value and standard deviation for three replicates of four different concentrations (n = 12), and three injections for each replicate.

Precision

System precision

System precision was assessed by analysis of ten replicates (n=10) of a standard mixture solution and three brands of tea sample solutions. The RSDs of peak area response in **Table 3** show the satisfactory repeatability of the system (<3 %).

Table 3. Precision data.

System precision (n=10)				
%RSD				
	Standard	**Sample1**	**Sample2**	**Sample3**
EGC	1.30	0.31	1.10	1.31
C	0.63	2.61	2.03	2.05
EGCG	0.96	0.45	1.05	1.79
EC	0.19	1.27	2.85	2.79
ECG	1.27	0.44	2.99	2.13
caffeine	1.29	0.42	1.11	0.71

Method precision (n = 6)	**Sample 1**		**Sample 2**		**Sample 3**	
	*content	%RSD	*content	%RSD	*content	%RSD
EGC	25.92	8.03	23.54	9.02	11.30	6.47
C	6.20	4.65	2.40	10.38	0.92	6.50
EGCG	49.21	9.93	18.97	11.68	8.98	8.74
EC	10.05	7.08	3.79	3.74	1.51	4.09
ECG	16.45	9.45	3.77	8.95	1.92	7.85
caffeine	23.46	9.54	21.54	6.32	10.52	6.79

Intermediate precision (n = 4)	**Sample 1**		**Sample 2**		**Sample 3**	
	*content	%RSD	*content	%RSD	*content	%RSD
EGC	21.29	11.52	20.87	10.72	10.88	9.32
C	4.96	10.54	2.08	9.88	0.84	7.71
EGCG	49.52	6.48	24.61	7.29	12.12	10.08
EC	8.53	10.82	3.45	11.13	1.54	8.80
ECG	15.55	7.36	4.05	10.41	1.89	11.82
caffeine	23.00	9.06	23.03	8.98	12.24	9.24

*Average content (mg per gram of dry weight of green tea sample), and three injections for each replicate.

Method precision and intermediate precision

Six replicates (n=6) of the three brands of green tea were analyzed in the same day to determine method precision or repeatability. Three brands of green tea were analyzed on four different days (n = 4) to evaluate intermediate precision. The percent relative standard deviations (RSD) of the assay results were determined. The RSDs shows the suitability of the method for determination of catechins and caffeine in green tea. The summary of precision results is listed in **Table 3**.

Quantitative analysis in green tea samples

A mixture of 5 catechins and caffeine from 30 commercial green tea products purchased in Thailand were analyzed by the validated method as described above. The content of individual catechins (EGC, C, EGCG, EC and ECG) and caffeine in samples are shown in **Table 4**. The major catechins are EGCG and EGC. Theophylline and theobromine were also monitored and no peak of both xanthines was detected in all tea samples.

Table 4: Five catechins and caffeine content in green tea infusion.

	(-) EGCG			(-) EGC			(-) EC			(-) ECG			(+) C			caffeine		
	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD	Mean	SD	% RSD
Products of Thailand																		
C. sinensis var. assamica																		
TH 01	30.64	0.53	1.73	14.54	0.36	2.50	4.85	0.14	2.87	7.03	0.54	7.71	0.49	0.04	7.66	22.68	1.24	5.45
TH 02	31.75	2.79	8.80	13.66	0.82	5.98	4.50	0.20	4.41	6.40	0.63	9.80	0.74	0.07	9.97	24.06	2.15	8.94
TH 03	25.38	1.14	4.50	12.28	1.00	8.11	2.19	0.19	8.88	4.84	0.28	5.75	0.52	0.03	6.05	20.16	1.29	6.40
TH 04	22.57	1.74	7.73	14.36	0.69	4.83	2.03	0.09	4.66	3.77	0.21	5.51	0.33	0.02	5.40	20.45	1.75	8.57
TH 05	18.97	2.22	9.68	23.54	2.12	9.02	3.79	0.14	3.74	3.77	0.34	8.95	2.40	0.25	9.38	21.54	1.36	6.32
TH 06	14.51	0.78	5.39	8.65	0.17	1.93	1.88	0.16	8.47	2.77	0.26	9.24	0.15	0.01	6.98	17.04	1.57	9.22
TH 07	13.77	0.70	5.12	12.14	0.90	7.42	1.66	0.15	9.25	1.89	0.17	8.96	0.25	0.02	9.29	9.68	0.55	5.70
Mean	22.51			14.17			2.99			4.35			0.70			19.37		
Total	**44.72**	**12.15**																
C. sinensis var. sinensis																		
TH 08	11.88	1.00	8.38	10.21	0.40	3.97	1.72	0.09	5.02	1.92	0.17	8.60	0.26	0.02	6.75	11.84	0.16	1.36
TH 09	10.52	0.67	6.33	7.96	0.25	3.16	1.35	0.02	1.83	1.69	0.15	9.01	0.30	0.02	6.40	8.06	0.13	1.58
TH 10	9.76	0.70	7.20	8.68	0.73	8.41	1.07	0.08	7.08	1.48	0.13	8.81	0.15	0.01	9.03	5.81	0.57	9.78
TH 11	8.98	0.78	8.74	11.30	0.73	6.47	1.51	0.06	4.09	1.92	0.15	7.85	0.92	0.06	6.50	10.52	0.71	6.79
TH 12	5.19	0.19	3.67	6.31	0.29	4.60	0.74	0.04	4.76	1.01	0.00	0.20	0.11	0.00	4.19	6.21	0.33	5.26
Mean	9.27			8.89			1.28			1.60			0.35			8.49		
Total	**21.39**	**4.91**																
Japanese "*sencha*" tea and packed in tea bag by Thai manufacturing																		
TH 13	46.65	4.89	9.93	45.92	2.08	8.03	10.05	0.71	7.08	16.45	1.55	9.45	6.20	0.29	4.65	23.46	2.24	9.54
TH 14	46.21	1.81	3.97	46.96	1.56	2.73	9.48	0.21	2.21	6.67	0.32	4.87	0.45	0.02	3.95	18.46	0.34	1.82
TH 15	45.52	1.04	2.28	46.52	1.94	4.16	7.65	0.41	5.30	7.06	0.46	6.47	0.57	0.01	1.31	20.18	0.69	3.42
Mean	45.87			46.67			9.06			10.06			2.41			20.70		
Total	**114.12**	**9.73**																
Products of Japan																		
Sencha																		
JP 01	58.21	4.13	7.09	49.12	1.03	2.10	10.69	0.52	4.82	11.83	0.93	7.90	1.92	0.13	6.77	26.66	0.55	2.08
JP 02	53.11	1.34	2.53	49.43	0.52	1.05	11.11	0.64	5.78	11.42	0.18	1.59	2.16	0.17	7.69	25.94	0.46	1.76
JP 03	44.47	1.29	2.90	52.48	1.63	3.11	11.58	0.39	3.41	8.05	0.31	3.87	0.65	0.03	5.23	22.88	0.92	4.02
JP 04	43.32	3.08	7.12	40.28	0.72	1.80	9.98	0.72	7.25	8.88	0.41	4.61	2.38	0.18	7.61	27.22	0.87	3.19
JP 05	40.02	0.47	1.18	45.64	1.23	2.70	10.44	0.21	2.03	7.58	0.34	4.54	1.00	0.02	2.05	20.12	0.19	0.97
JP 06	38.95	1.02	2.63	40.75	1.48	3.64	8.40	0.47	5.55	8.01	0.39	4.84	0.84	0.05	5.43	23.92	0.45	1.89
JP 07	23.59	1.82	7.71	34.98	2.98	8.53	6.92	0.36	5.22	5.33	0.35	6.51	0.09	0.01	9.71	15.44	1.14	7.38
Mean	43.10			44.67			9.87			8.73			1.29			23.17		
Total	**107.66**	**20.57**																
Bancha																		
JP 08	20.15	1.37	6.78	24.04	0.92	3.81	4.93	0.28	5.69	4.87	0.30	6.06	0.10	0.01	8.69	14.69	0.96	6.56
JP 09	17.73	1.18	6.65	30.58	1.08	3.52	6.00	0.29	4.77	4.78	0.32	6.74	0.20	0.01	5.33	12.52	0.20	1.60
JP 10	7.55	0.40	5.34	14.82	1.02	6.88	2.93	0.05	1.80	2.21	0.10	4.42	0.13	0.01	8.13	8.29	0.56	6.74
Mean	15.14			23.15			4.62			3.95			0.14			11.83		
Total	**47.01**	**16.97**																
Products of China																		
Bi-Luo-Chun																		
CH 01	33.90	1.04	3.07	9.28	0.58	6.30	2.83	0.11	3.92	8.26	0.34	4.08	6.10	0.27	4.40	27.62	0.61	2.19
CH 02	32.07	1.05	3.28	5.40	0.42	7.76	3.73	0.31	8.38	8.33	0.34	4.09	4.78	0.29	6.09	22.84	1.10	4.80
Mean	32.99			7.34			3.28			8.30			5.44			25.23		
Total	**57.34**	**4.29**																
Long jing																		
CH 03	21.65	1.08	4.98	4.35	0.09	2.14	2.49	0.16	6.61	5.35	0.38	7.06	4.35	0.25	5.71	20.95	0.19	0.89
CH 04	18.93	0.65	3.45	5.77	0.23	4.00	1.71	0.09	5.39	3.70	0.29	7.74	1.45	0.11	7.80	19.84	0.95	4.78
CH 05	13.20	0.96	7.24	2.80	1.26	5.09	4.17	0.35	8.36	4.80	0.27	9.53	0.21	0.02	9.23	9.07	0.91	9.99
Mean	17.93			4.31			2.79			4.62			2.00			16.62		
Total	**31.64**	**6.51**																

Mean = average content of each catechin and caffeine in mg per gram of green tea (dry weight), mean value for six replicates and three injections for each replicate.

Total = average content of total 5 catechins in mg per gram of green tea (dry weight),

Products of Thailand

It was observed that Thai green tea produced from variety *assamica* presented significantly higher content of individual catechins than those from variety *sinensis* ($p< 0.05$). In variety *assamica*, EGCG was the catechin presented at the highest level (13.77-30.64 mg/g dry tea), followed by EGC, ECG, EC and C. In contrast, variety *sinensis*

contained catechins with a trend of EGCG ≥ EGC > EC > ECG > C. The content of EGCG (5.19-11.88 mg/g dry tea) was not significant different from EGC(6.31-11.30 mg/g dry tea) (p> 0.05). Variety *assamica* contained 9.68-24.06 mg/g of caffeine which was significant different from that measured in variety *sinensis* (5.81-11.84 mg/g). These suggest that tea variety has a great impact to the variation in the levels of the individual catechins and caffeine.

The composition of catechins in Thai tea brands manufactured from Japanese *sencha* raw materials was also studied. The results showed that these samples had a significantly higher level among individual catechins than those from both Thai tea varieties (p> 0.05). However, the level of caffeine of these raw materials was similar to that of variety *assamica*.

Products of Japan

Japanese *sencha* samples showed the highest contents of EGCG and EGC in the range from 25.59 to 58.21 and 34.98 to 52.48 mg/g dry tea, respectively. EC, ECG and C found in sencha samples ranged from 6.92-11.58, 5.33-11.83 and 0.09-2.38 mg/g dry tea, respectively. The results showed the similar high levels among individual catechins between these *sencha* products and *sencha* raw materials established above, indicating the high quality control during tea manufacturing. In comparison with *bancha* samples, *sencha* samples had a significantly higher level among individual catechins (p> 0.05). In *bancha* samples, the level of EGCG (7.55-20.15 mg/g dry tea) was lower than EGC (14.82-30.58 mg/g dry tea). EC, ECG and C found ranged from 2.93-6.00, 2.21-4.87 and 0.10-0.20 mg/g dry tea, respectively. All Japanese samples showed an overall similar tendency among individual catechins (EGC > EGCG > EC > ECG > C). The content of caffeine also differed between two grades of Japanese green tea. *Bancha* samples had significantly lower level of caffeine compared to *sencha* samples.

Products of China

Among two brands of Chinese green tea, *biluochun* samples presented the higher level of each catechin than that of *longjing*. EGCG was a major constituent (between 13.20-33.90 mg/g dry tea) in both types, followed by ECG and EGC. It is noteworthy that more of C was presented in *biluochun* samples than other green tea samples (4.78-6.10 mg/g dry tea) with overall trend of EGCG > ECG > EGC > C > EC. In contrast, the level of C in *longjing* samples was lower than EC. The highest amounts of caffeine were found in *biluochun* samples (22.84-27.62 mg/g dry tea) while *longjing* samples showed the lower level (9.07-20.95 mg/g dry tea).

Comparison of Chinese, Japanese and Thai green teas

The green tea analysis results in this study show large variations among tea from different brands. It was established that Japanese *sencha* samples contained the highest amounts of total catechins followed by Chinese *biluochun*, Japanese *bancha*, Thai variety *assamica*, Chinese *longjing* and Thai variety *sinensis* (**Figure 2**).The average amount of individual catechins and caffeine in each sample group is also illustrated in **Figure 2**. EGCG and EGC, the major antioxidant in green tea, were found in the highest quantity in Japanese sencha. The level of EGCG in Thai variety *assamica* was significantly lower than those of Japanese *sencha* and Chinese *biluochun* but higher than those of Japanese *bancha* and Chinese *longjing* (p< 0.05). EGC content found in Thai variety *assamica* was significantly lower than those of Japanese *sencha* and *bancha* but higher than those of Chinese *biluochun* and *longjing* (p< 0.05). It was noteworthy that the sum of EGCG and ECG contents in Thai variety *assamica* was similar to that of Japanese *bancha*. EC was also significantly higher in *sencha* samples. The high amount of ECG was found in the *sencha* and *biluochun* samples in a similar level. C was found in larger quantity in Chinese tea; *biluochun*. The order of caffeine content presented in tea sample was Chinese *biluochun* > Japanese *sencha* >Thai variety *assamica* >Chinese *longjing* > Japanese *bancha* >Thai variety *sinensis*. The levels of caffeine in samples were between 5.81-27.62 mg/g dry tea. This range caused little adverse effects in healthy adults[22]. All these results may be explained by the different varieties, cultivation condition and manufacturing processes.

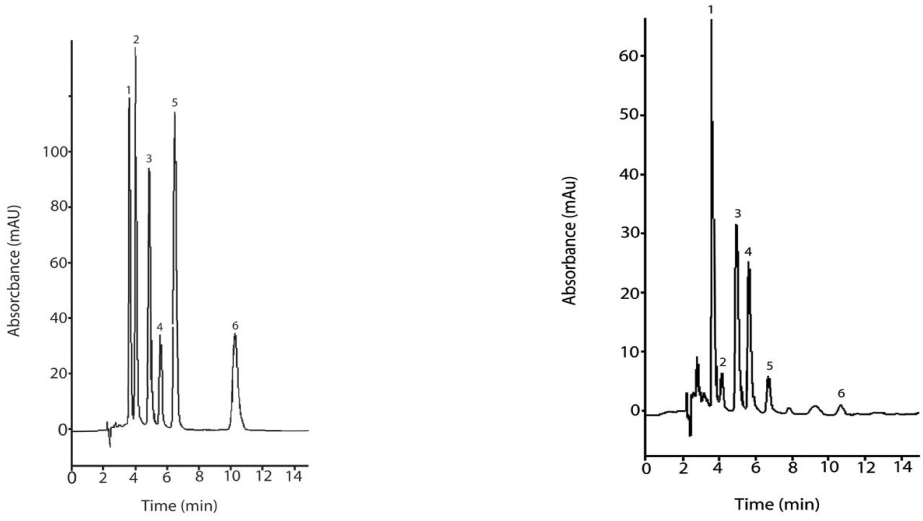

Figure 1.HPLC chromatogram of (A) five standard catechins (at concentrations of 60-70 µg/ml) and standard caffeine (at a concentrations of 30 µg/ml) and (B) a green tea sample. Peaks; 1: (-) epigallocatechin (EGC); 2: (+) catechin (C); 3: (-) epigallocatechin gallate (EGCG); 4: caffeine; 5: (-) epicatechin (EC); and 6: (-) epicatechin gallate (ECG).

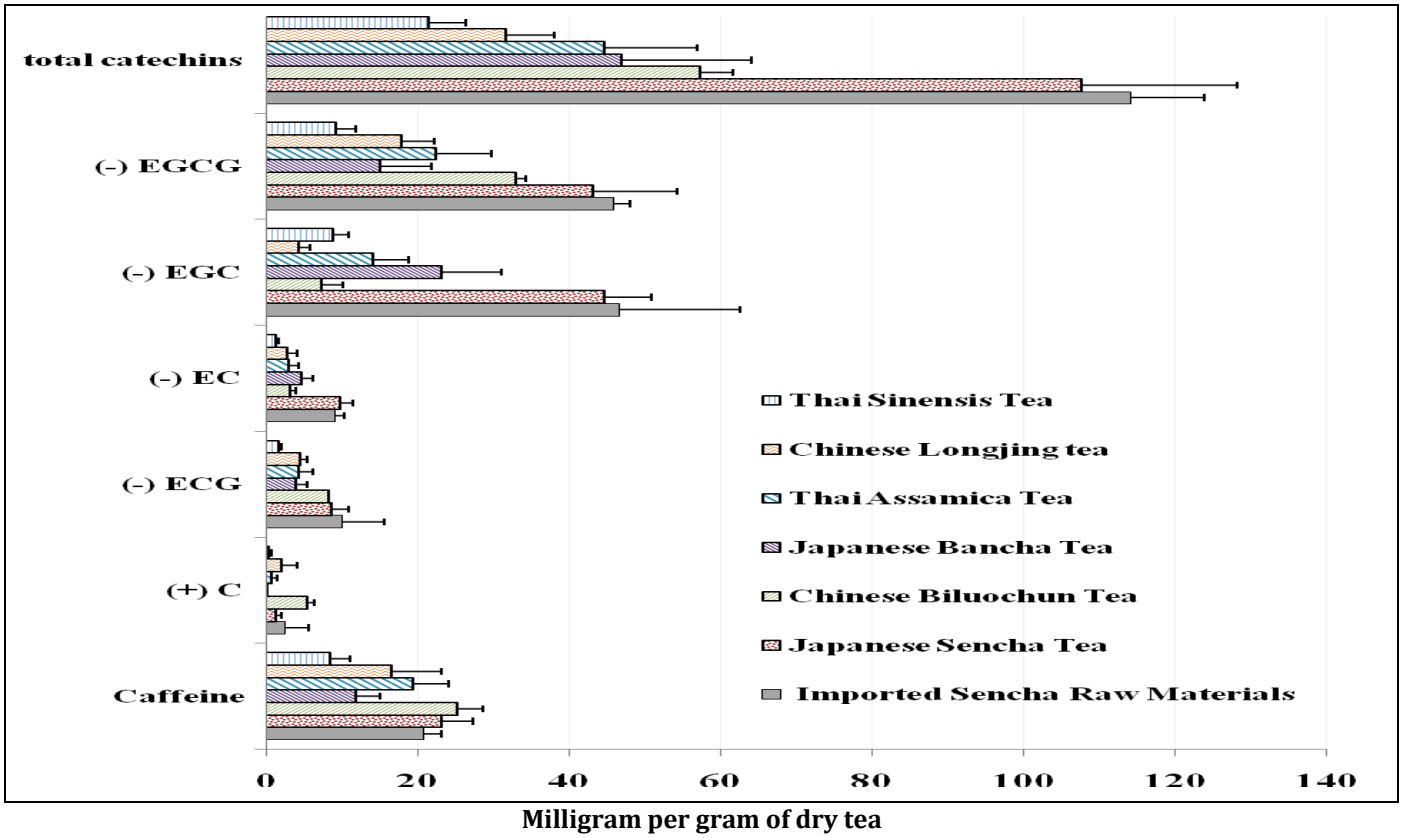

Figure 2. Average contents of individual catechins, total catechins and caffeine in green tea samples; 7 Thai variety *sinensis* teas, 5 Thai variety *assamica* teas, 3 imported *sencha* raw materials, 7 Japanese *sencha* teas, 3 Japanese *bancha* teas, 2 Chinese *biluochun* teas and 3 Chinese *lonjing* teas. The vertical bars represents mean±SD in gram of constituent per gram of dry tea.

Conclusion:

Among green tea products commercialized in Thailand, quality of tea products in this study has shown wide variations among countries and among within countries due to species and manufacturing method. Thai variety *assamica* was found to have higher level of five individual catechins and caffeine than variety *sinensis*. In comparison with products from Japan and China, Thai variety *assamica* had similar level of total catechins to Japanese bancha but higher than those of Chinese longjing however its caffeine content was similar to Japanese sencha. Nows a days, there is no labelling legislation that would require tea companies to report the levels of catechins and caffeine in their products. These data of the catechins and caffeine content among various brands would exhibit useful information for consumer and manufacturers to select teas with the highest content of beneficial compounds for health-promoting and suitable raw materials for extracting catechins.

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