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Screening of Epigallocatechin-3-Gallate (EGCG) Content in *Camellia sinensis* Products

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Abstract

Epidemiological studies have proven the influences of tea consumption in prevention of chronic diseases. Tea (*Camellia sinensis*) is a major source of epigallocatechin-3-gallate (EGCG) compound with pharmacological properties such as anti-obesity, anti-diabetes, anti-inflammation. The aims of this study is to analyze and compare the EGCG content in commercialized tea-based products, infusion tea and ready-to-drink tea beverages available in the local market. This study is divided into two phases. The first phase involved the extraction of EGCG compound from four types of tea namely as white tea (WT), green tea (GT), oolong tea (OT) and black tea (BT) using aqueous and methanol extraction techniques. The amount of EGCG is determined using HPLC. The second phase involved the characterization of EGCG compound in infusion tea bags (ITB) and ready-to-drink tea beverages (RTD) using HPLC. Our findings recorded that GT contain the highest EGCG concentration in comparison to other tea types using both methanol and aqueous extraction methods. Temperature and extraction time have influence on the extraction yield. This study also revealed that ready-to-drink tea beverages contains significantly lower level of EGCG compared to the infusion tea hence makes ready-to-drink beverages less healthy for consumption especially for obese and diabetics individuals.

Keywords: Tea; EGCG; TLC; HPLC; Pharmacological properties; Infusion tea

Introduction

Tea is one of the most preferable beverages among people throughout the world due to its beneficial effect towards human health. There are four major types of tea has been derived from *Camellia sinensis* leaves known as White tea (WT), Green tea (GT), Oolong tea (OT) and Black tea (BT) which differ in processing step. The compound responsible to provide positive effects of tea is known as EGCG, a tea catechin [1,2]. Different types of tea have different level of tea catechin [3]. The pharmacological properties of EGCG such as antioxidant, anti-obesity, anti-diabetes and anti-inflammatory makes tea as potential alternative for disease control, weight management and regulating cholesterol level [4-6].

In the market, there have been an increased number of health supplements and food products desired from tea claims to help in reducing body weight, lowering the blood cholesterol level and blood glucose level. However, the level of EGCG content may differ in different types of tea products hence, the pharmacological effect might be different. Therefore, this study presents a method to characterize the presence of EGCG in several commercialize tea-based product in order to validate its effectiveness as food supplement. This study also reveal the optimize condition to extract EGCG compound from different types of tea samples. Results from this study provide insights in empowering consumer rights and ensure the food security.

Materials and Methods

Materials and reagents

Fourteen infusion tea bag samples and twelve ready-to-drink samples were purchased randomly from the market. ECGC standard were purchased from Calbiochem with HPLC purity not less than 99.5%. HPLC grade of Acetonitrile, methanol, acetic acid were used during extraction and HPLC analysis.

Extraction procedure

5 g of the tea sample was brewed in 250 ml methanol and 250 ml distilled water at temperature 37°C for 60 minutes with continuous stirring. The tea solution was filtered using Whatman No.1 filter paper twice. Next, the extract solution was centrifuged at 4500 rpm for 3 minutes. The supernatant was collected into conical flask and the residue was discarded. After that, the solution was dried using freeze dryer until powder is obtained. The dried extract was stored in -20°C refrigerator. This method was repeated for GT and BT samples. The process was repeated twice.

Optimization of aqueous extraction condition

For optimization of temperature, 5 g of tea sample was brewed in 250 ml distilled water at different temperature (29°C, 50°C, 60°C and 80°C) for 60 minutes with continuous stirring. The procedure was similar as mention in Extraction procedure. The optimum temperature determined from each types of tea is used as temperature for optimization of extraction time, 5 g of tea sample was brewed in 250 ml distilled water at different extraction time (20 min, 30 min, 40 min and 60 min). The temperature was set based on the optimum

temperature of the desired tea samples. The extraction procedure was conducted as mention in previous section. This method was repeated twice for WT, GT, OT and BT samples.

HPLC analysis

Qualitative and quantitative determination of EGCG was performed using High Performance Liquid Chromatography (HPLC). The chromatographic separations were carried out using Agilent C-18 column (4.6 × 150 mm, 5 micron). The combination of 20% buffer A (water with 0.05% Trifluroacetic Acid (TFA)) and 80% buffer B (acetonitrile with 0.05% TFA) were used as the mobile phase and filtered using 0.20 µm nylon filter. The flow rate was set at 1.0 ml/min with oven temperature maintained at 25°C. Detection with diode array detector was used at wavelength 280 nm. EGCG standard (99.5% purity) was prepared in serial dilutions to generate a standard curve. The samples were prepared by diluting 5 mg of freeze-dried extract into 1 ml of buffer A and filtered with 0.20 µm nylon filter. For infusion tea and ready-to-drink tea, 1 ml of undiluted tea solution from each samples was tested. The presence of EGCG in tea extracts and tea samples were analyzed by comparing the chromatogram of the samples to the chromatograms of the EGCG standard while EGCG concentration from each sample was calculated using EGCG standard curve. The results were presented in mean with standard deviation.

Results and Discussion

Extraction of EGCG from GT and BT using methanol extraction and aqueous extraction method

Methanol extraction yield a higher EGCG concentration compared to aqueous extraction in both GT and BT samples. The similar result was obtained in previous study by Vuong et al. [7], extraction with methanol provide a better efficiency for catechin extraction compared to extraction with hot water only (aqueous extraction). Since EGCG is water soluble compound, it has higher solubility in water compares to organic solvent such as methanol, but it has higher polarity towards methanol which influenced it to be soluble in methanol (Table 1) [8].

Extraction method	Tea type	Extraction yield (%)	EGCG Concentration (mg/ml)
Methanol Extraction	GT	8.21	0.9347 ± 0.08
	BT	1.84	0.5385 ± 0.12
Aqueous Extraction	GT	26.87	0.6705 ± 0.09
	BT	20.46	0.4410 ± 0.12

Table 1: Comparison of EGCG extraction process using methanol extraction and aqueous extraction method.

Methanol extraction (ME) yield higher EGCG concentration compare to aqueous extraction (AQ) at the same extraction condition, 37°C and 60 minutes extraction continuous with continuous stirring. The result was presented with mean, n=2 and standard deviation.

The presence of EGCG in tea samples were analyzed by comparing the retention time of tea samples to EGCG standard through HPLC results. The EGCG standard showed one sharp and high peak at retention time 2.762. The tea samples showed 2 single peaks at retention time 2.740 and 3.091. Based on the EGCG standard reference, the peak formed at retention time 2.740 minutes was indicated as EGCG while peak second peak obtained at 3.091 minutes was referred as caffeine according to Vasisht et al. [9].

EGCG standard curve is plotted with R^2>0.9882 to quantify the amount of EGCG is the tea samples. High level of EGCG concentration is recorded in GT samples (1.0958 ± 0.14) followed by WT (0.9604 ± 0.00), OT (0.7747 ± 0.05) and BT (0.7747 ± 0.05). This result is in accordance with Chen et al. [1], Yi et al. [10], Demir et al. [11] which reported EGCG is abundantly found in GT.

Optimization of temperature and extraction time in extraction of EGCG from WT, GT, OT and BT using aqueous extraction method

Higher EGCG concentration is obtained using methanol extraction, however, in this study, aqueous extraction method is chosen to be optimized since water is the solvent usually used to prepare a cup of tea for daily consumption.

Extraction of EGCG by aqueous extraction is influenced by the temperature and extraction time [7,8,12]. From the experiment, the extraction of EGCG from WT, GT and OT are influenced by the temperature. As illustrated in Figure 1, there are a significant different between the EGCG concentration for WT, GT and OT at different temperature and showed that 80°C yield the highest EGCG concentration for WT (0.9301 ± 0.04), GT (0.8835 ± 0.09) and OT (0.6949 ± 0.04). However, the highest EGCG concentration for BT samples is obtained at lower temperature with EGCG concentration value of (0.4949 ± 0.02). This finding mimic previous reports by Vasisht et al. [9], Pastoriza et al. [13] and Vuong et al. [14] which 80°C is the best temperature to yield higher concentration of EGCG content in tea sample especially for green tea and black tea is not affected by temperature [8].

WT and GT samples were affected by the extraction time. Based on Figure 1, the concentration of EGCG increased with time for WT but inversely proportional for GT. This finding mimic previous reports where the yield of EGCG from tea increases as the extraction time increases [8]. Meanwhile, the results for GT is in synchrony with the results obtained by Pastoriza et al. [15] which indicates that the yield of EGCG from GT decrease with time. However, the amount of EGCG obtained from OT and BT are almost similar with highest EGCG concentration was extracted at 40 minutes.

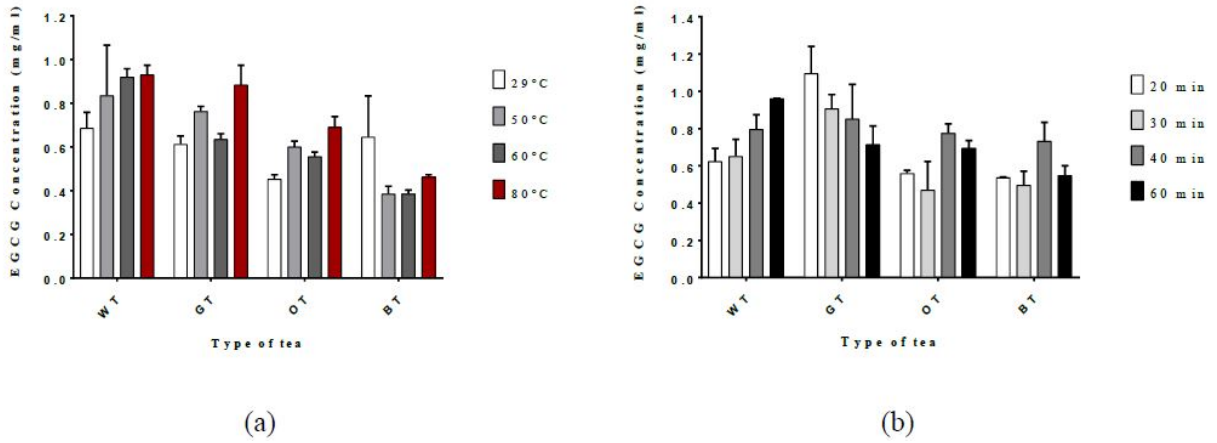

Figure 1: Optimization of aqueous extraction condition for extraction of EGCG from WT, GT, OT and BT. (a) Temperature optimization, (b) extraction time optimization. The result is presented with mean (n=3) and standard deviation.

When comparing the level of EGCG in infusion tea bag (ITB) and ready-to-drink tea beverages (RTD), the EGCG concentration in ITB is much higher compared to RTD. EGCG and other catechin counterparts are easily disrupted by heat, light and chemicals which makes EGCG content in RTD beverages is lesser along the manufacturing process rather than ITB which undergo simple fermentation process. The preparation of infusion tea suggested by manufacturer is brewing the tea in boiling water for 3 minutes may facilitated the extraction of more catechin. The result is in agreement with Oliveira [15] where by EGCG content in ITB is higher compare to RTD beverages.

Besides, different brands of tea products contain different level of EGCG content which is depends on the processing methods hence the price reflect the quality of the tea brand in the market. Based on Table 2, there are a significant different between the level of EGCG in different brands of WT, GT, OT and BT products which reflects its value for health. Same results were obtained in Table 3 where the difference among the brands is significant in GT and BT products.

Types of tea	Brands	Number of sachets/mg	Price/number of sachets (RM)	EGCG concentration (mg/ml)
WT	A	1/2 mg	29.90/25	0.2246 ± 0.034
	B	1/1.8 mg	24.50/20	0.4715 ± 0.057
GT	C	1/2 mg	5.40/25	0.7222 ± 0.082
	D	1/2 mg	11.60/25	0.8741 ± 0.249
	E	1/2 mg	8.90/25	0.4619 ± 0.252
	F	1/2 mg	9.75/25	0.7106 ± 0.076
OT	G	1/5 mg	24.00/52	0.3516 ± 0.057
	H	1/2 mg	15.30/25	0.3737 ± 0.020
	F	1/2 mg	9.75/25	0.7496 ± 0.004
BT	I	1/2 mg	3.10/20	0.2626 ± 0.097
	J	1/2 mg	6.50/25	0.3750 ± 0.087
	K	1/2 mg	2.30/20	0.2668 ± 0.034
	D	1/2 mg	2.70/25	0.2845 ± 0.025

	E	1/2 mg	10.55	0.3719 ± 0.049

Table 2: EGCG concentration in ready-to-drink tea beverages. The result is presented with mean (n=3) and standard deviation.

Types of tea	Brands	Price per 250 ml (RM)	EGCG concentration (mg/ml)
WT	N	2.50	0.0762 ± 0.007
GT	L	2.50	0.1534 ± 0.005
	M	1.80	0.3196 ± 0.010
	N	2.50	0.2967 ± 0.004
	O	1.90	0.1825 ± 0.002
	P	2.20	0.1564 ± 0.040
OT	N	2.50	0.1182 ± 0.008
BT	L	2.50	0.0503 ± 0.004
	M	1.80	0.2626 ± 0.008
	N	2.50	0.1982 ± 0.031
	O	1.90	0.1048 ± 0.001
	P	2.20	0.1358 ± 0.006

Table 3: EGCG concentration in ready-to-drink tea beverages (n=3).

Conclusion

In conclusion, the optimum extraction condition of white tea, green tea, oolong tea and black tea is different from each other. The different was influenced by the fermentation step during tea processing. GT contain higher EGCG content compare to the other types of tea, WT, OT and BT. The extraction of EGCG compound from WT, GT and OT were influenced by temperature but not for BT. 80°C is the best temperature for to extract EGCG from WT, GT and OT using aqueous extraction. Apart from that, higher EGCG content is extracted at shorter time for GT but longer time is needed for WT. Both EGCG from OT and BT samples were best extracted at 40 minutes.

The level of EGCG content per ml is much lower compare to infusion tea hence, it provide less benefit for health. Besides, different brands of tea products contain different EGCG content depends on the processing methods hence the price reflect the quality of the tea brand in the market. Ready-to-drink tea beverage is an unhealthy option for drinks. Therefore, it is recommended to drink infusion tea especially GT at least three cups per day to get the benefits of EGCG towards health. However, further study and research are needed to evaluate the effectiveness of tea consumption in treatment and prevention of obesity for normal, overweight and obese people.

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