Malaysian Journal of Chemistry, 2023, Vol. 25(5), 57-62
(Special Issue: *The 4th International Conference on Chemistry and Material Sciences 2022*)

The Analysis of Catechin and Caffeine in Green Tea Capsules and Teabags using High-Performance Liquid Chromatography

Amirah Najihah Mohd Affandi, Wan Nazihah Wan Ibrahim, Hairul Amani Abdul Hamid, Noraini Hamzah and Nursyamsyila Mat Hadzir*
Universiti Teknologi MARA, Shah Alam, 40450 Shah Alam, Selangor, Malaysia
*Corresponding author (e-mail: nursyamsyila@uitm.edu.my)

Green tea leaves contain antioxidants such as catechin and caffeine, which can speed up metabolism, and helps break down excess fat. However, there are concerns that overconsumption of catechin and caffeine could cause liver toxicity and kidney failure, respectively, to the consumers. This study involved the quantitative analysis of catechin and caffeine in three different brands of green tea teabag and two different brands of green tea capsule using High-Performance Liquid Chromatography (HPLC). All standards were extracted using methanol, dissolved in the mobile phase of deionized water and acetonitrile (30:70 v/v), analysed at 270 nm in triplicates using the HPLC-DAD system. The data interpretation was conducted using standard calibration plot with correlation coefficient for catechin and caffeine of 0.9823 and 0.9856, respectively. Teabag samples were labelled A, B, and C, while capsules samples were labelled D and E. The highest amount of catechin (7274.2679 mg) and caffeine (2253.8581 mg) was found in Sample E. In Sample D, only 1.1561 mg of caffeine was detected and no catechin was detected which probably due to high level of detection (LOD) and quantification (LOQ). The analysis of catechin and caffeine using HPLC was proven to be reliable, simple, sensitive, and rapid.

Keywords: Green tea; caffeine; catechin; high-performance liquid chromatography

Received: September 2022; Accepted: December 2022

Tea from *Camellia sinensis* L. leave is widely consumed after plain water. Tea is consumed worldwide, with China, India, and Kenya being the major tea producers. Approximately three billion kilograms of tea was produced and consumed worldwide per year. From the literature, tea was documented to be unintentionally found by a Chinese Emperor, Shen Nung in 2737 BCE when some tea leaves fell into boiling water, and producing a very pleasant fragrance [1]. Tea is categorized to green tea, oolong tea, and black tea, based on the degree of fermentation and level of antioxidants [2]. Green tea is a famous herbal plant of Chinese tea with abundant health benefits, mainly attributed to its polyphenol content thus making it a very popular beverage [3]. Most of the green tea polyphenols are flavonols, commonly known as catechins. The main products derived from green tea are extracts in the form of liquid or powder, which vary in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%) [4].

Caffeine and catechin have been reported by many researchers for their health-promoting properties to human [5]. Caffeine could improve memory, mental functioning and alertness, as well as decrease fatigue. Catechin exhibits benefits such as anti-inflammatory, antiarthritic, anticarcinogenic, anti-cancerous, anti-mutagenic, antibacterial, antiviral, antifungal, anti-coccidial, antiprotozoal, antiparasitic, anti-infective, hypocholesterolaemia, resistance to capillary blood congestion, and hypolipidemic effects [5]. In comparison to apples, red grapes, chocolates, and red wine, green tea is the most prevalent source of caffeine and catechins among all dietary sources. It was reported that approximately 2% - 5% caffeine present in green tea leaves and 71 mg of catechin in epigallocatechin gallate present in 100 mL of green tea [2, 6].

Catechin and caffeine in green tea have been reported could speed up the metabolism of the body especially catechin by breaking down the excess fat [7]. The clinical use of green tea has been proven to stimulate the weight loss process for overweight or obese people [8], which led to the production of green tea capsules for weight loss management. It was reported that green tea supplements positively impacted weight loss and weight management even in small amounts due to the presence of either catechins or caffeine [9]. However, there are concerns that overconsumption of catechin could cause liver toxicity and iron deficiency anemia to the consumer [10]. Excessive amount of caffeine due to overconsumption of green tea also lead to various adverse effects such as headache, irregular heartbeat, and kidney failure. A high caffeine intake may increase the risk of congenital disabilities and miscarriage [11].

Initially, this study was proposed based on concerns due to the trend to lose weight quickly, which resorts to taking green-tea products, especially green

†Paper presented at the 4th International Conference on Chemistry and Material Sciences 2022 (IC2MS2022)

tea capsules without knowing the adverse effects they could cause. Hence, the consumer needs to be aware of the amount of green tea intake to avoid the negative effects of consuming green tea. It is essential to consumers and manufacturers to know the safe amount of caffeine and catechin within the permissible limit of 65mg of caffeine per 12 liquid ounces in beverages, 200mg in pills, and 300mg catechin for healthy adults [12]. In addition, HPLC is chosen as the analytical tool for analyzing caffeine and catechin as it a simple, rapid, and precise method with an economical mobile phase for the simultaneous separation and quantification of analytes. Therefore, the present study was carried out to determine the amount of caffeine and catechin in three different brands of green tea teabag and two different brands of green tea capsules using HPLC.

EXPERIMENTAL

Chemicals, Reagent and Samples

The chemicals and reagents that have been used in the present work were acetonitrile and methanol of the HPLC grade (Merck, Germany), caffeine of 98.5% purity and (+)-catechin of 98% purity were purchased from Fisher chemicals, Belgium. The samples were purchased from hypermarket and pharmacy in Shah Alam, Selangor as well as from the online shopping platform. The samples were three different brands of green tea teabag (labeled as Samples A, B and C) and two different brands of green tea capsule (labeled as Samples D and E). The commercial name of the samples are kept confidential.

Preparation of the Standard Solutions of Catechin and Caffeine

The sample preparation of catechin and caffeine was carried out by following the experimental procedure by Oliveira [13] with some modifications. Both standards were prepared at various concentrations (50, 100, 150, 200 and 250 ppm) using methanol in 50 mL volumetric flasks.

Preparation of the Samples

The preparation of sample was carried out by following the experimental procedure by Oliveira [13] with some modifications. Firstly, the tea leaves and powder were taken out from the teabag and capsule, respectively. Approximately 50 mg of each sample was weighed and placed in a 50 ml volumetric flask. Then, methanol was added to the mark, shook well to extract both catechin and caffeine from the samples. Next, each solution was filtered using a filter paper into their respective sample bottles. Further filtration was carried out using 0.45 µm Durapore membrane and finally, 1 µL of the filtrate was injected into the HPLC.

HPLC Instrumentation Condition

The chromatographic analysis was performed on an Agilent Technologies® High Performance Liquid Chromatography (HPLC) system with separation was carried out using C18 column (15 cm x 4.6 mm) at oven temperature of 25 °C, with UV detection at 270 nm and flow rate of 1 mL/minute. The mobile phase used for isocratic elution was 30% deionized water and 70% acetonitrile.

RESULTS AND DISCUSSION

Analysis of Catechin Standard Solutions and Its Calibration Curve

A series of standard catechin (50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm) was measured at 270 nm wavelength using HPLC-DAD and they were prepared in triplicate. The average retention time for standard catechin was found to be between 1.318 and 1.319 min, as shown in Table 1. The average peak area was recorded from each standard solution, and the calibration curve was constructed by plotting recorded peak areas versus the concentration of standard catechin, as shown in Figure 1. From the plotted calibration curve, the linear equation obtained was $y = 0.4279x + 8.2592$ with a correlation coefficient (R^2) value of 0.9823.

Table 1. The retention time and peak area for different concentration of standard catechin.

Concentration of standard (ppm)	Average Peak Area (mAU*s)	Average retention time (min)
50	34.9456	1.318
100	47.0320	1.318
150	67.0025	1.318
200	96.3164	1.318
250	117.6247	1.319

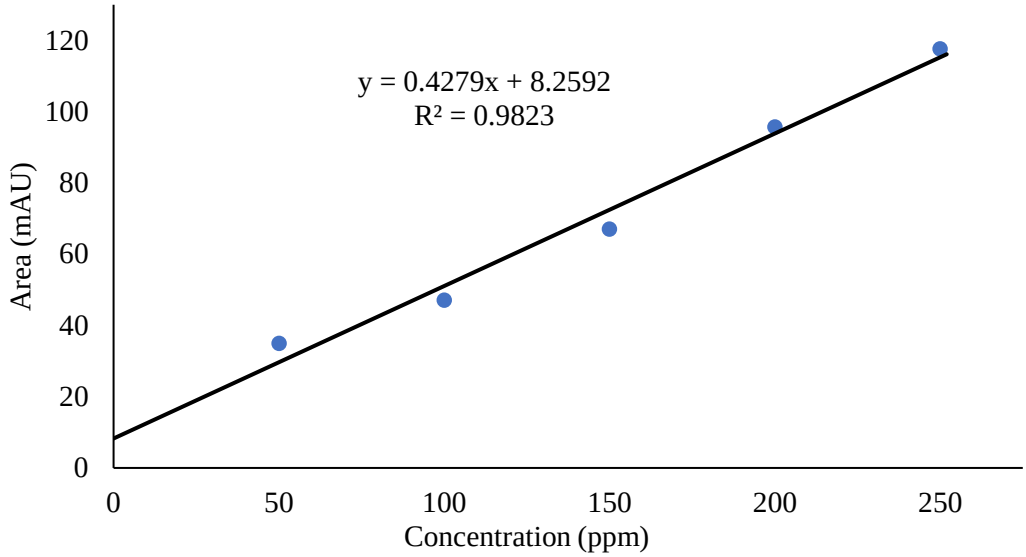

Figure 1. Calibration curve of catechin standard at 270 nm.

Table 2. The retention time and peak area for different concentration of standard caffeine.

Concentration of standard (ppm)	Average Peak Area (mAU*s)	Average retention time (min)
50	179.7353	1.443
100	283.0768	1.441
150	424.4147	1.442
200	641.0350	1.442
250	753.4117	1.444

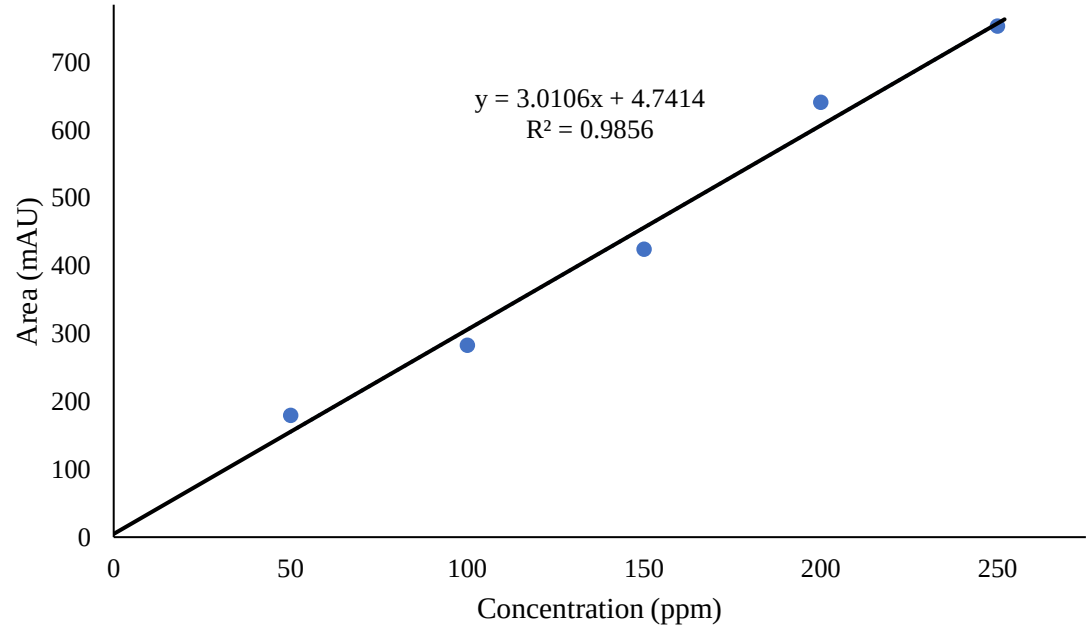

Figure 2: Calibration curve of caffeine standard at 270 nm.

Table 3: The LOQ and LOD values of catechin and caffeine in green tea capsule samples.

Compound	Regression equation	Standard Error of Intercept	Slope	LOD (ppm)	LOQ (ppm)
Catechin	y=0.4279x + 8.2592	5.5068	0.4279	42.4686	128.6926
Caffeine	y=3.0106x + 4.7414	34.8758	3.0106	38.2283	115.8432

Analysis of Caffeine Standard Solutions and Its Calibration Curve

A series of standard caffeine (50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm) were measured at wave-length of 270 nm using HPLC-DAD in triplicate. The retention time obtained for standard caffeine was found to be in the range between 1.441 and 1.444 min as shown in Table 2. The peak area obtained from each standard was recorded and the calibration curve was constructed by plotting recorded peak areas versus concentration of standard caffeine as shown in Figure 2. From the plotted calibration curve, the linear equation obtained was y = 3.0106x + 4.7414 with correlation coefficient (R^2) value of 0.9856.

Limit of Detection and Limit of Quantitation

Limit of detection (LOD) measure the smallest concentration of standards compound that can be detected. Limit of quantification (LOQ) quantifies the lowest level of catechin and caffeine standards that can be precisely and accurately detected. Both were determined using the formulas LOD = 3.3 x SE/S and LOQ = 10 x SE/S. According to Little [14], S is the slope of the regression line and SE stands for standard error, derived from the data analysis in MS Excel. Regression analysis can easily determine the calibration curve's standard error (SE), making it the most straight-forward technique [15]. As they relate to the sensitivity of the appropriate analytical method, determining the limit of detection (LOD) and quantification (LOQ) is crucial. Table 3 illustrates the LOD and LOQ results.

Catechin and Caffeine Content in Green Tea Samples

The amount of catechin in the green tea teabag and capsule samples were calculated using the equation obtained from the calibration curve of standard catechin. Table 4 shows the amount of catechin in teabag samples, with the highest amount of catechin was found in Sample A (221.2046 mg), followed by Sample C (145.2162 mg) and Sample B (124.4894 mg). According to Dekant [12], catechin intake in the form of green tea infusions should be safe up to the maximum consumption of 734 mg EGCG/person/day. The catechin content in teabag samples is less than 734 mg, which can be considered safe for daily consumption. However, for Sample A, the consumers should limit the intake to 3 serving/day due to the high content of catechin in the sample.

From Table 4, it can also be seen that catechin was not detected in Sample D, which probably due to its small amount, which is below the limit of detection (LOD) and limit of quantification (LOQ) as discussed in the previous section. Even so, the manufacturer labelled on the packaging that catechin is present in Sample D. On the contrary, Sample E has the highest amount of catechin recorded (7274.2679 mg) among all the samples tested (both teabags and capsules). The high amount of catechin in Sample E is quite alarming as Dekant [12] proposed that a tolerable upper intake level of 300 mg EGCG/person/day for food supplements.

Table 4: The catechin content in green tea samples

Sample	Retention time (min)	Peak Area (Mau*s)	Concentration ± RSD (ppm)	Weight of catechin in 50 mg sample (mg)	Weight of catechin in teabag sample (mg)
A	1.288	52.7894	104.0669 ± 3.05	5.2033	221.2046
B	1.256	36.0832	65.0245 ± 1.19	3.2512	124.4894
C	1.287	39.3422	72.6408 ± 0.42	3.632	145.2162
D	not detected	not detected	not detected	not detected	not detected
E	1.282	42.4267	7984.9264 ± 0.88	399.2463	7274.2679

The equation derived from the calibration curve of the standard caffeine was used to calculate the amount of caffeine in the green tea teabag and capsule samples. Table 5 shows the highest calculated amount of caffeine in Sample A (28.1111 mg) (green tea teabag), followed by Sample C (26.2708 mg) and Sample B (17.8573 mg). A study by Batool [16] stated that green tea contains 60mg of caffeine. Therefore, the amount of caffeine in all teabag samples are within the permissible limit. Consequently, sample A, with the highest caffeine concentration, can harm the consumer if the green tea is taken more than twice daily. A study by Garg [17] reported that the high concentration of caffeine in green tea preparation might be due to long brewing time at high temperature.

The amount of caffeine in Sample D was found to be 1.1561 mg, while Sample E contained 2253.8581 mg of caffeine as tabulated in Table 5. Most people experience no behavioral effects with less than 300 mg of caffeine [16]. As a result, the amount of caffeine in Samples A, B, C and D were found to be within the permissible limit (300 mg) hence they can be considered as safe for multiple servings per day. On the other hand, prolong consumption of Sample E may cause serious side effects to the consumers due to the excessive amount of caffeine (2253.8581 mg).

Validation of the Analytical Method

Table 6 shows the percentage of relative standard deviation (%RSD) of catechin and caffeine. %RSD value indicates the precision of the closeness of the value obtained by replicating measurements under specified conditions. Based on the %RSD value, the deviation of the sample value from the mean value can be measured. The lowest value of %RSD from the catechin sample was found in Sample C (0.42), while the highest %RSD amount was found in Sample A (3.05). The low %RSD indicates that the measurement was precisely carried out. Hence, the result for sample C is more precise than sample A. The %RSD value of caffeine for Sample D showed that it was the least precise measurement as compared to the other measurements for other samples.

CONCLUSION

The amount of both catechin and caffeine content in all green tea samples were successfully identified. The highest amount of catechin (7274.2679 mg) and caffeine (2253.8581 mg) was found in Sample E followed by Samples A, C and B. Sample D was found to have the least amount of caffeine (1.1561 mg) with no catechin detected which probably due to the high level of LOD and LOQ.

Table 5. The caffeine content in green tea samples.

Sample	Retention time (min)	Peak Area (Mau*s)	Concentration ± RSD (ppm)	Weight of caffeine in 50 mg sample (mg)	Weight of caffeine in teabag sample (mg)
A	1.441	44.5567	13.225 ± 0.41	0.6613	28.1111
B	1.441	32.8224	9.3274 ± 0.84	0.4664	17.8573
C	1.443	44.3046	13.1413 ± 0.43	0.6571	26.2708
D	1.442	14.6046	3.2761 ± 1.33	0.1638	1.1561
E	1.446	79.2251	2474.0484 ± 0.51	123.7024	2253.8581

Table 6. Percentage of relative standard deviation (%RSD) of catechin and caffeine content.

Sample	Catechin sample (%RSD)	Caffeine sample (%RSD)
A	3.05	0.41
B	1.19	0.84
C	0.42	0.43
D	not detected	1.33
E	0.88	0.51

ACKNOWLEDGEMENTS

The authors would like to extend our gratitude to the Dean and management of Faculty of Applied Sciences, Universiti Teknologi MARA for allowing Amirah Najihah Mohd Affandi to use the chemicals, instruments and laboratory facilities to complete her laboratory work as partial fulfillment of the requirements for the Degree of Bachelor of Science (Hons.) Applied Chemistry.

REFERENCES

1. Wheeler, D. S. & Wheeler, W. J. (2004) The Medicinal Chemistry of Tea. *Drug Development Research*, **61(2)**, 45–65.

2. Prasanth, M., Sivamaruthi, B., Chaiyasut, C. & Tencomnao, T. (2019) A Review of the Role of Green Tea (Camellia sinensis) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy. *Nutrients*, **11(2)**, 474.

3. Saeed, M., Naveed, M., Arif, M., Kakar, M. U., Manzoor, R., Abd El-Hack, M. E., Alagawany, M., Tiwari, R., Khandia, R., Munjal, A., Karthik, K., Dhama, K., Iqbal, H. M. N., Dadar, M. & Sun, C. (2017) Green tea (Camellia sinensis) and Ltheanine: Medicinal values and beneficial applications in humans—A comprehensive review. *Biomedicine and Pharmacotherapy*, **95**, 1260–1275.

4. Chacko, S. M., Thambi, P. T., Kuttan, R. & Nishigaki, I. (2010) Beneficial effects of green tea: a literature review. *Chinese medicine*, **5**, 13.

5. Musial, C., Kuban-Jankowska, A. & Gorska-Ponikowska, M. (2020) Beneficial Properties of Green Tea Catechins. *International Journal of Molecular Sciences*, **21(5)**, 1744.

6. Dufresne, C. J. & Farnworth, E. R. (2001) A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry*, **12(7)**, 404–421.

7. Westerterp-Plantenga, M. S. (2010) Green tea catechins, caffeine and body-weight regulation. *Physiology and Behavior*, **100(1)**, 42–46.

8. Jurgens, T. M., Whelan, A. M., Killian, L., Doucette, S., Kirk, S. & Foy, E. (2012) Green tea for weight loss and weight maintenance in overweight or obese adults. *Cochrane Database of Systematic Reviews*, **12**.

9. Hursel, R., Viechtbauer, W. & Westerterp-Plantenga, M. S. (2009) The effects of green tea on weight loss and weight maintenance: a meta-analysis. *International Journal of Obesity*, **33(9)**, 956–961.

10. Fan, F. S. (2016) Iron deficiency anemia due to excessive green tea drinking. *Clinical Case Reports*, **4(11)**, 1053–1056.

11. Morgan, S., Koren, G. & Bozzo, P. (2013) Is caffeine consumption safe during pregnancy? *Canadian Family Physician*, **59**, 361–362.

12. Dekant, W., Fujii, K., Shibata, E., Morita, O. & Shimotoyodome, A. (2017) Safety assessment of green tea based beverages and dried green tea extracts as nutritional supplements. *Toxicology Letters*, **277**, 104–108.

13. Oliveira, R. M. M. De. (2012) Quantification of catechins and caffeine from green tea (Camellia sinensis) infusions, extract, and ready-to-drink beverages. *Food Science and Technology*, **32(1)**, 163–166

14. Little, T. (2015) Method Validation Essentials, Limit of Blank, Limit of Detection, and Limit of Quantitation, *BioPharm International*, **28(4)**.

15. Vial, J. & Jardy, A. (1999) Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. *Analytical Chemistry*, **71(14)**, 2672–2677.

16. Batool, M. (2018) Determination of Caffeine in Soft and Energy Drinks Available in Market by UV. *Archives of Nanomedicine: Open Access Journal*, **1(2)**, 35–40.

17. Garg, A. K. (2021) Quantitative Analysis of Caffeine in the Green Tea, Black Tea and Soft Drink Using UV-Visible Spectrophotometer. *Indian Journal of Science and Technology*, **14(37)**, 2860–2864.