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Original Article

Unveiling the total catechin profile in tea leaves: a novel one-step extraction method empowered by UPLC-IDX-Orbitrap mass spectrometry

Revelando o perfil total de catequinas em folhas de chá: um novo método de extração em uma etapa capacitado pela espectrometria de massa UPLC-IDX-Orbitrap

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Abstract

More catechins are found in green tea than in any other type of tea, with its predominant production taking place in Asian nations. Consumption of green tea has been strongly correlated with a reduced risk of many diseases. This study introduces a new, efficient, and reliable method for extracting total catechins using ultra-high-performance liquid chromatography coupled with an ID-X-Orbitrap Mass spectrometer (UHPLC-IDX-Orbitrap-MS). The method was then applied to quantify the catechin content in green tea, yielding results comparable to previously published studies. Among the various sources of green tea analyzed, the lowest average catechin content was observed in Vietnam, Japan (2: Matcha), and Morocco, ranging between 346 and 322 mg/L. Conversely, the highest average catechin content (between 424 and 422 mg/L) was found in Sri Lanka and Japan (1: Sencha). For the remaining green tea extracts, the catechin levels ranged from 367 to 410 mg/L, exhibiting similar values. These findings demonstrate the high reproducibility of the proposed extraction procedure, with a relative standard deviation (RSD) error of less than 15% for the catechin standard. Additionally, the limit of detection for catechins was determined to be 1 ng mL^{-1}. This study serves as a pilot investigation for extracting catechins from various green tea sources. Future research will focus on identifying all active compounds present. Furthermore, it is worth noting that this study aligns with the goals set forth in Saudi Vision 2030, which aims to diversify the country's economy and promote scientific advancements in various fields, including healthcare and agriculture.

Keywords: active compounds, green tea, UHPLC, mass spectrometer, analysis.

Resumo

Mais catequinas são encontradas no chá verde do que em qualquer outro tipo de chá, com sua produção predominante ocorrendo nos países asiáticos. O consumo de chá verde tem sido fortemente correlacionado com a redução do risco de muitas doenças. Este estudo apresenta um método novo, eficiente e confiável para extrair catequinas totais usando cromatografia líquida de altíssima performance acoplada a um espectrômetro de massa ID-X-Orbitrap (UHPLC-IDX-Orbitrap-MS). O método foi aplicado para quantificar o conteúdo de catequina no chá verde, produzindo resultados comparáveis a estudos publicados anteriormente. Entre as diversas fontes de chá verde analisadas, o menor teor médio de catequina foi observado no Vietnã, Japão (2: Matcha) e Marrocos, variando entre 346 e 322 mg/L. Por outro lado, o teor médio mais elevado de catequina (entre 424 e 422 mg/L) foi encontrado no Sri Lanka e no Japão (1: Sencha). Para os demais extratos de chá verde, os níveis de catequina variaram de 367 a 410 mg/L, apresentando valores semelhantes. Esses achados demonstram a alta reprodutibilidade do procedimento de extração proposto, com um erro de desvio-padrão relativo (RSD) inferior a 15% para o padrão de catequina. Além disso, o limite de detecção para catequinas foi determinado em 1 ng mL-1. Este estudo serve como uma investigação-piloto para a extração de catequinas de várias fontes de chá verde. A investigação futura centrar-se-á na identificação de todos os compostos ativos presentes. Além disso, é importante notar que este estudo está alinhado com os objetivos estabelecidos na Visão Saudita 2030, que visa diversificar a economia do país e promover avanços científicos em vários campos, incluindo saúde e agricultura.

Palavras-chave: compostos ativos, chá verde, UHPLC, espectrômetro de massa, análise.

1. Introduction

Tea, derived from the *Camellia sinensis* plant, is one of the most widely consumed beverages globally. Its cultivation dates back thousands of years in Southeast Asia, with Chinese legend attributing its discovery to Emperor Shen Nung in 2737 BC (Mukhtar and Ahmad, 1999). Camellia, belonging to the Theaceae family, was originally believed

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to be indigenous to Yunnan Province in China and northern Myanmar (Lu et al., 2021; Tchamgoue et al., 2023).

Tea is classified into six major types based on oxidation and fermentation techniques (Chang, 2015). The processing methods, particularly drying and fermenting, determine the final type of tea (Reygaert, 2018). China's tea production has witnessed significant growth in response to rising domestic demand, supported by the country's robust economy, which has grown at an annual rate of 9% over the past three decades. The rapid development of herbal tea beverages in China, a nation with a long history of tea consumption, has further contributed to this increase (FAO, 2022).

Green tea, a non-fermented variety, contains higher amounts of catechins compared to black or oolong tea (Cabrera, Artacho et al., 2006). Among the various tea polyphenols present in green tea, green tea catechins (GTCs) are the most abundant, constituting 30-40% of the extractable solids in dried green tea leaves (Yang and Landau, 2000; Chen et al., 2017). The primary catechins found in green tea are (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Cabrera et al., 2006). Chen et al. (1995) ranked the catechins as follows: EGCG > ECG > EGC > EC > C, with EGCG being the most abundant and active. Additionally, tea contains a significant amount of caffeine (3-5%), which remains unaffected by various processing methods (Chu, 1997).

Furthermore, as scientific evidence supporting the health benefits of tea continues to accumulate, its popularity as a widely-consumed beverage is on the rise. Researchers are exploring the use of natural plant products, including green tea (*Camellia sinensis*), which has long been used in traditional Chinese medicine, to combat infectious diseases, potentially leading to significant cost savings in healthcare (Reygaert, 2018). Green tea has been found to lower blood pressure, total cholesterol, and the risk of coronary heart disease (Chen et al., 2017), as well as obesity (Reygaert, 2018). Its antioxidant, antimutagenic, antidiabetic, anti-inflammatory, antibacterial, antiviral, and particularly anti-inflammatory properties contribute to the prevention of periodontal and oral diseases, promoting dental health when consumed daily (Rashidinejad et al., 2026). Consumption of at least 100 milliliters (approximately half a cup) of green tea per day has shown potential for reducing the prevalence of depression and dementia by lowering levels of the stress hormone cortisol (Reygaert, 2018). Regular consumption of green tea has also been inversely associated with the incidence of influenza and fever-related illnesses in school-aged children (Wang et al., 2008; Noda et al., 2012). However, for these catechins to exert their beneficial effects in the body, they must be bioaccessible after ingestion. Once inside the body, catechins undergo metabolic processing in the liver, small intestine, and colon, resulting in the formation of glucuronide and sulfate conjugates or methyl epicatechins (Reygaert, 2018).

Green tea has the potential to counteract the detrimental effects of environmental toxins by preventing significant DNA, protein, and cell damage (Chen et al., 2017), thereby reducing the toxicity associated with common environmental toxicants such as pesticides, smoking, mycotoxins, PCBs, and arsenic. In addition to its therapeutic applications, green tea consumption is beneficial for infection prevention (Reygaert, 2018). Green tea extracts or pure catechins can be incorporated into cheese and other dairy products to enhance the absorption of these antioxidant compounds in the diet (Rashidinejad et al., 2016).

The catechin content in green tea is influenced by factors such as geographical location, growing conditions, harvesting time, leaf processing, and brewing temperature and duration. Consequently, there is substantial variation in catechin content among different types and brands of green tea (Reygaert, 2018). Wang et al. (2008) reported that higher brewing temperatures led to a decrease in catechin concentrations but an increase in catechin isomers. Optimal brewing conditions were found to be 85 °C for 3 minutes, resulting in a peak EGCG concentration of 50.69 mg/100 ml and maximum sensory scores (Saklar et al., 2015).

The aim of this investigation is to develop a cost-effective method to measure the total catechins in different types of green tea from around the world. We also aim to use advanced technology to accurately identify the specific chemical composition of these catechins. This research aligns with Saudi Vision 2030, which promotes research and innovation in the field of food science and nutrition, and aims to enhance healthier lifestyles and sustainable agriculture.

2. Materials and Methods

2.1. Sample collection

Ten tea brands of raw unprocessed green tea samples from around the world (Figure 1) were collected from the market in October 2023. They were as follows; 1) Vietnam, 2) Indonesia, 3) India, 4) Morocco, 5) Japan (1: Sencha, Fujiyama Province), 6) Japan (2: Matcha), 7) China (1:Sencha, Zheiang Province), 8) China (2:Wu Lu, Misty mountain Province of North Fujian) and 9) Sri Lanka, 10) Uzbekistan (Figure 2).

2.2. Solvents preparation and grinding procedure

For M1: Methyl-tert-butyl ether / methanol (3:1, v/v) solvent; 75 mL methyl-tert-butyl ether with 25 mL of methanol was Mix in an appropriate container, then kept in the freezer at -20 ^{0}C. Whereas, M2: Methanol / water (3:1, v/v) was prepared with 75 mL LC-MS water and 25 mL of methanol then mixed in an appropriate container, later kept in the fridge at 4 ^{0}C. Tea samples was milled mechanically using milling device (SPEX™ SamplePrep 2010 Geno/Grinder (Matyash et al., 2008; Emwas et al., 2015; Salem et al., 2016).

2.3. Extraction procedure

50 mg of dried tea samples was weighted, then 1 mL of pre-cooled M1 solvent was add to the dried sample, vortex for 1 min at 2000 rpm, then Incubate and sonicate the sample for 45 min at 4 ^{0}C. For phase separation, 650 µL of solvent M2 was add to each sample and vortex for 1 min. then the samples was centrifuge at 4 ^{0}C,

Figure 1. Locations of various green tea samples around the world. The map was produced using Datawrapper (2024).

14000 rcf for 10 min, 200 µL of polar phase (lower layer) was transferred to Cert Amb-ID vial for the analysis of targeted secondary metabolites (Matyash et al., 2008; Salem et al., 2016). Moreover, Catechin, Natural flavonoid compound (ab142852) was used as a standard from abcam, CAS Number: 154-23-4 and Purity: > 98%. And water was purified by a water purification system (Thermo Scientific™, USA). Methanol (LC/MS optima grade) (Jeong et al., 2020).

2.4. Mass spectrometer

Three mass analyzers are included in the Orbitrap ID-X mass spectrometer. It was utilized to determine the m/z of the molecules under investigation. The Orbitrap IDX spectrometer has a high resolution (> 120,000) and a low mass error (3 ppm). Negative-mode electrospray ionization (ESI-) The mass spectrometer was calibrated using a "Calibration Mix ESI (Thermo ScientificTM)" that could be purchased and followed the manufacturer's instructions.

2.5. Ultra-High Pressure Liquid Chromatography (UHPLC)

The samples were separated using a C18 column (Acquity CSH 100 x 2.1 mm, 1.7 µm) that was automatically infused (5 µl each) through the UHPLC system. The flow rate was 0.5 mL/min, and the separation was done using the following gradient: A: 100 percent water, 0.1 percent formic acid, and B: 100 percent acetonitrile + 0.1 percent formic acid made up the mobile phase solvents. Table 1 and Figure 3 summarize the gradient elution program. To separate the catechin and caffeine analytes, the final UHPLC settings and gradient elution protocol were optimized (Salem et al., 2016).

2.6. Standards preparation and quantification

A serial dilution process of the stock solution (ng/mL) was used to make working standard solutions with concentrations of 1, 10, 50, 100, 500, 1000, and 10000 pg/mL.

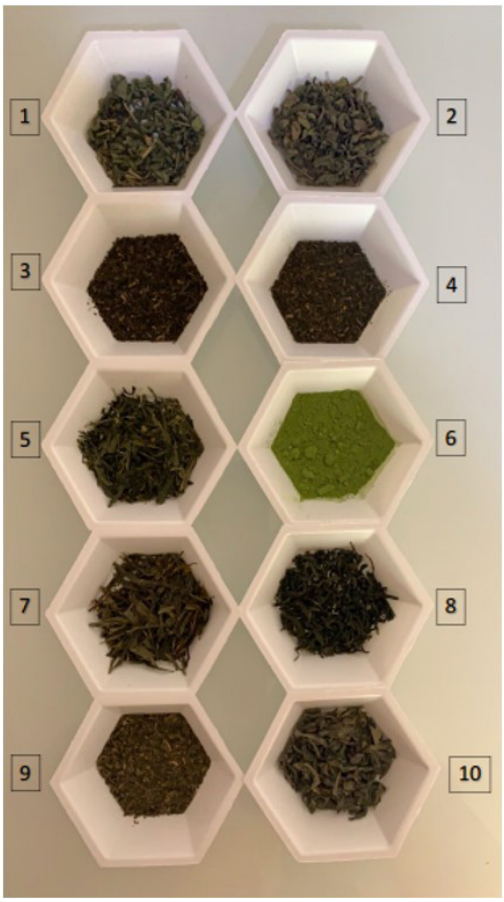

Figure 2. Variety of green tea samples selected for this research are as the following: 1) Vietnam, 2) Indonesia, 3) India, 4) Morocco, 5) Japan (1: Sencha, Fujiyama Province), 6) Japan (2: Matcha), 7) China (1: Sencha, Zheiang Province), 8) China (2: Wu Lu, Misty mountain Province of North Fujian) and 9) Sri Lanka, 10) Uzbekistan.

The quantification was prepared by creating regression curves for each of the catechin standards that were studied (Table 2). Quan, Xclalibur software (Thermo ScientificTM)

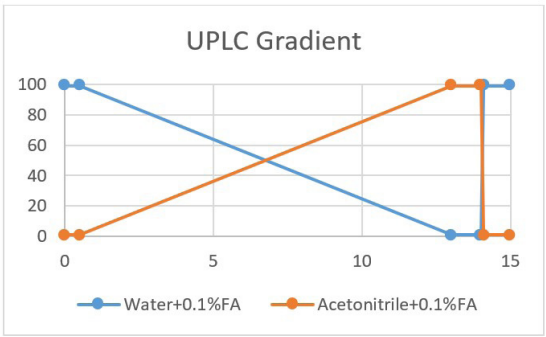

Figure 3. Ultra-high pressure liquid chromatography gradient.

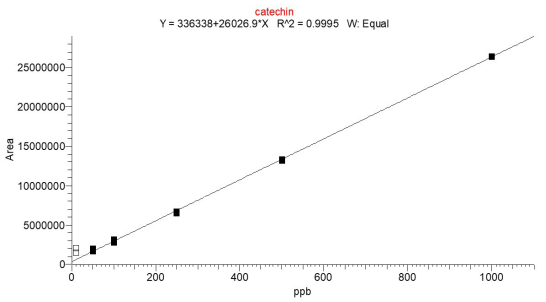

Figure 4. Regression cure of catechin standards (10, 50, 100, 250, 500, and 1000) ppb.

Table 1. HPLC – gradient elution program.

Time (min)	Flow (ml/min)	%B	Curve
0		**Run**	
0.5	0.5	5	5
13	0.5	99	5
14	0.5	99	5
14	0.5	5	5
15	0.5	5	5
15		Stop Run	

%B refres to the percentage of the mobile phase B in the solvent composition.

data processing was used to construct the standard curves. The linearity over the six ranges yielded R^2 values that were appropriate. Each analyte's limit of detection (LOD) and limit of quantitation (LOQ) values were calculated using concentrations comparable to three times and ten times the signal-to-noise ratio.

2.7. Statistical analysis

Statistical analyses, including descriptive statistics, as well as, Tukey HSD post-hoc test was performed to assess the pairwise differences. All analyses were carried out using JMP Pro 13, a computer program for statistics developed by the Statistical Analysis System (SAS) Institute.

3. Results and Discussion

The total concentration of catechin molecules was determined by measuring the peak area and utilizing a calibration standard regression curve based on the concentration of pure catechin (Figure 4). The limit of quantification (LOQ) and the limit of detection (LOD) were determined using a signal-to-noise ratio of 50 and 10, respectively (Table 2 and Figure 4). The extraction method showed higher efficiency for samples with higher concentrations of caffeine, similar to previous experiments.

Within a short time frame of less than 5 minutes (Figure 5), the four catechins studied were successfully separated using UPLC-IDX Orbitrap-MS. The identified catechins and their respective retention times (RT) were as follows: (-)-epicatechin (EC) at m/z 289.07089 Da (RT: 1.46 min), (-)-epicatechin-3-gallate (ECG) at m/z 441.08140 Da (RT: 3.41 min), (-)-epigallocatechin (EGC) at m/z 305.06575 Da (RT: 1.19 min), and (-)-epigallocatechin-3-gallate (EGCG) at m/z 457.07626 Da (RT: 1.56 min).

In this study, sample number 6 from Japan (Matcha) exhibited the lowest concentration among all the measured green tea samples (Figure 2). The UHPLC-IDX-Orbitrap-MS analysis of the extracts from ten green tea samples enabled the quantification of total catechins by investigating the presence of four catechin molecules: epicatechin, epigallocatechin, epicatechin-3-O-gallate, and epigallocatechin-3-O-gallate (Figure 5).

The total catechin content in all the measured green tea samples exhibited higher concentrations in samples from Sri Lanka and Japan (1; Sencha). The concentration order was as follows: China (2; Fujian), India, Indonesia, China (1; Fujiyama), Uzbekistan, Vietnam, Japan (2: Matcha), and Morocco (R2 = 0.95, F = 19.9, P < 0.0001, Tukey HSD posthoc test, P< 0.05) (Figure 6 and Table 3). Supplementary Material SP provides additional information on the analysis.

Table 2. LOD, LOQ, and R^2 values for the calibration curves (n = 6) generated from Thermo Scientific™ UHPLC–IDX-Orbitrap/MS instrument for the caffeine compound.

Component Name	Curve Index	Weighting Index	Equation	LOD (µg/mL)	LOQ (µg/mL)
catechin	Linear	Equal	$Y = 336338 + 26026.9 * XR\hat{}2 = 0.9995$	1	50

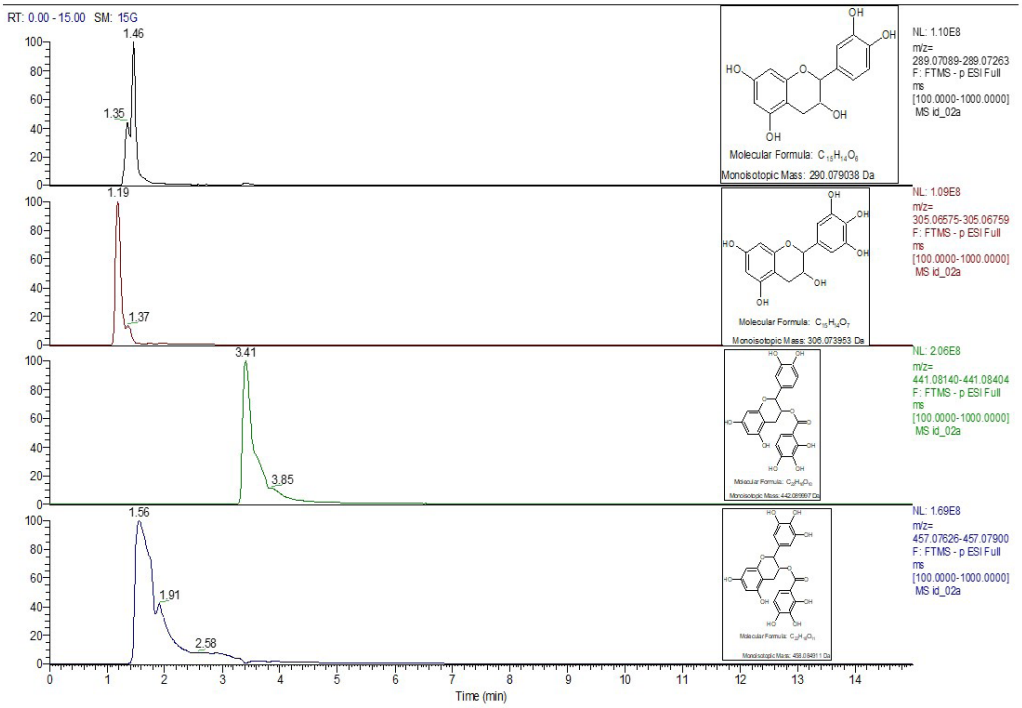

Figure 5. Extracted ESI (-) MS chromatogram of the four main catechins in tea: a: (-)-epicatechin (RT:1.46min) at m/z,289.07089 Da. b: (-)-epigallocatechin (RT:1.19min) at m/z,305,06575 Da. c: (-)-Epicatechin-3-O-gallate (RT:3.41min) at m/z,441.08140 Da. d: (-)-epigallocatechin -3-O-gallate (RT:1.56min) at m/z,457.07626. Da.

Table 3. A summary of the concentration of catechin in (mg/L; PPM) in the various samples of the green tea samples from the samples around the world: 1) Vietnam, 2) Indonesia, 3) India, 4) Morocco, 5) Japan (1: Sencha, Fujiyama Province), 6) Japan (2: Matcha), 7) China (1: Sencha, Zheiang Province), 8) China (2: Wu Lu, Misty mountain Province of North Fujian) and 9) Sri Lanka, 10) Uzbekistan. Letters in means represents significant differences according (Tukey HSD multiple comparison post-hoc test, $P < 0.05$).

Samples	Mean ± SD	Range	Variance	Std Err	CV
China (1)	367.80±3.11 ^{bc}	4.40	9.69	2.20	0.85
China (2)	409.97 ±4.23^{ab}	5.98	17.90	2.99	1.03
India	408.82±4.71 ^{ab}	6.66	22.16	3.33	1.15
Indonesia	408.68±7.23 ^{ab}	10.23	52.32	5.11	1.77
Japan (1)	421.97±8.12^{a}	11.48	65.91	5.74	1.92
Japan (2)	324.11±5.55 ^{c}	7.85	30.83	3.93	1.71
Morocco	322.76±1.48 ^{c}	2.10	2.21	1.05	0.46
Sri Lanka	424.37±4.08^{a}	5.77	16.66	2.89	0.96
Uzbekistan	367.44±1.55 ^{bc}	2.19	2.39	1.09	0.42
Vietnam	346.44±36.85 ^{c}	52.11	1357.66	26.05	10.64

Std Dev (standard deviation), Std Err (standared error), CV (coefficient of variation).

A typical brewed green tea beverage contains approximately 50-100 mg of catechins per 250 mL (Jówko, 2015). In the study, the results ranged from 300 to 400 mg/L, which corresponds to an average of 0.4 mg/mL when compared to 50 mg of extract, equivalent to 100 mg in a standard 250 mL beverage. Moreover, the study determined a total catechin concentration ranging from 0.3 to 0.4 mg/mL towards 50 mg of tea extract. Other studies have reported similar concentrations, ranging from 0.8 mg/mL towards 150-250 mg of tea extract in Bigelow Green Tea, to 0.7 mg/mL towards 150-250 mg in Celestial Seasoning Decaf Green Tea,

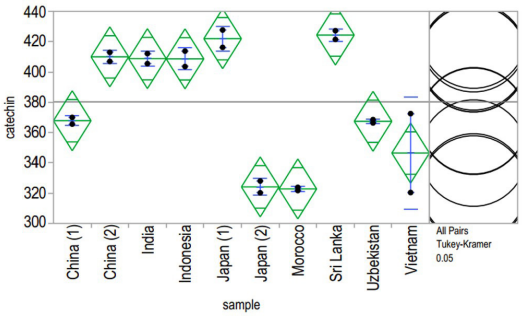

Figure 6. Oneway analysis of catechin by various green tea samples, blue color is mean and standard deviation, green is mean and anova.

and up to 2.2 mg/mL towards 150-250 mg in Celestial Seasoning Green Tea (Henning et al., 2003). Therefore, the extraction experiment yielded approximately twice the catechin content per milligram compared to the study by Henning et al. (2003) when recalculating the amount.

Another example can be seen in Koch et al. (2018), where a concentration of 3.63 mg/mL was reported in a 10-gram sample of Chinese green tea, corresponding to approximately 20-fold higher concentration compared to the study, which yielded around 0.36 mg/mL in 1 mg. It is worth noting that the concentration of bioactive compounds in green tea can vary significantly depending on preparation methods such as water temperature and steeping time (Rains et al., 2011).

4. Conclusion

This study demonstrates a robust method utilizing a fast extraction technique and fast high-resolution mass spectrometry (MS) to identify and characterize total catechins in tea samples. The developed method has the potential to be extended to the analysis of other natural extracts for targeted compound identification. Remarkably, within a timeframe of less than 5 minutes (Figure 5), all catechins were successfully separated and identified using UPLC-IDX Orbitrap-MS. Furthermore, the targeted analytical technique employed in this study proves to be a quick and accurate approach for determining catechin levels in 10 different green tea samples. Analysis of various green tea samples from around the world revealed distinct catechin concentrations, with significantly higher levels observed in samples from Japan (Sencha) and Sri Lanka compared to others. This pilot study provides a comparative analysis of catechin content in different green tea types. Future research will explore the complete extract and all compounds present in the samples to uncover additional active chemicals. Additionally, the findings of this research align with the objectives set forth in Saudi Vision 2030. By utilizing advanced analytical techniques and investigating the composition of green tea, this study contributes to the promotion of research and innovation in the field of food science and nutrition, supporting the goals of healthier lifestyles and sustainable agriculture.

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References

CABRERA, C., ARTACHO, R. and GIMÉNEZ, R., 2006. Beneficial effects of green tea: a review. *Journal of the American College of Nutrition*, vol. 25, no. 2, pp. 79-99. http://doi.org/10.1080/07315724.2006.10719518. PMid:16582024.

CHANG, K., 2015. *World tea production and trade Current and future development*. FAO.

CHEN, L., MO, H., ZHAO, L., GAO, W., WANG, S., CROMIE, M.M., LU, C., WANG, J.-S. and SHEN, C.-L., 2017. Therapeutic properties of green tea against environmental insults. *The Journal of Nutritional Biochemistry*, vol. 40, pp. 1-13. http://doi.org/10.1016/j.jnutbio.2016.05.005. PMid:27723473.

CHU, D. -C., 1997. Green tea-its cultivation, processing of the leaves for drinking materials, and kinds of green tea. In: T. YAMAMOTO, L.R. JUNEJA, D.-C. CHU, M. KIM, eds. *Chemistry and applications of green tea*. New York: CRC Press, pp. 1-11.

DATAWRAPPER [online], 2024 [viewed 14 May 2024]. Available from: https://www.datawrapper.de

EMWAS, A.-H.M., AL-TALLA, Z.A. and KHARBATIA, N.M., 2015. Sample collection and preparation of biofluids and extracts for gas chromatography–mass spectrometry. In: J.T. BJERRUM, ed. *Metabonomics: methods and protocols*. New York: Humana Press, pp. 75-90. http://doi.org/10.1007/978-1-4939-2377-9_7.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO, 2022. *Current global market situation and emerging issues*. FAO.

HENNING, S.M., FAJARDO-LIRA, C., LEE, H.W., YOUSSEFIAN, A.A., GO, V.L. and HEBER, D., 2003. Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity. *Nutrition and Cancer*, vol. 45, no. 2, pp. 226-235. http://doi.org/10.1207/S15327914NC4502_13. PMid:12881018.

JEONG, H., PARK, D.H., SEO, H.G., CHOI, M.-J. and CHO, Y., 2020. Effect of roasting time and cryogenic milling on the physicochemical characteristics of dried ginseng powder. *Foods*, vol. 9, no. 2, 223. http://doi.org/10.3390/foods9020223. PMid:32093227.

JÓWKO, E., 2015. Green tea catechins and sport performance. In: M. LAMPRECHT, ed. *Antioxidants in sport nutrition*. Boca Raton: CRC Press, pp. 123-140.

KOCH, W., KUKULA-KOCH, W., KOMSTA, Ł., MARZEC, Z., SZWERC, W. and GŁOWNIAK, K., 2018. Green tea quality evaluation based on its catechins and metals composition in combination with chemometric analysis. *Molecules*, vol. 23, no. 7, 1689. http://doi.org/10.3390/molecules23071689. PMid:29997337.

LU, K., SAN, K.K. and NYEIN, A.M., 2021. Background history of tea cultivation in Myanmar. In: R. DURIGHELLO, R. CURRIE and M. LUENGO, eds. *Tea landscapes of Asia: a thematic study*. Charenton-le-Pont: International Council on Monuments and Sites, pp. 343-366.

MATYASH, V., LIEBISCH, G., KURZCHALIA, T.V., SHEVCHENKO, A. and SCHWUDKE, D., 2008. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. *Journal of Lipid Research*, vol. 49, no. 5, pp. 1137-1146. http://doi.org/10.1194/jlr.D700041-JLR200. PMid:18281723.

MUKHTAR, H. and AHMAD, N., 1999. Mechanism of cancer chemopreventive activity of green tea. *Proceedings of the Society for Experimental Biology and Medicine*, vol. 220, no. 4,

pp. 234-238. http://doi.org/10.1046/j.1525-1373.1999.d01-40.x. PMid:10202395.

RAINS, T.M., AGARWAL, S. and MAKI, K.C., 2011. Antiobesity effects of green tea catechins: a mechanistic review. *The Journal of Nutritional Biochemistry*, vol. 22, no. 1, pp. 1-7. http://doi.org/10.1016/j.jnutbio.2010.06.006. PMid:21115335.

RASHIDINEJAD, A., BIRCH, E.J. and EVERETT, D.W., 2016. Green tea catechins suppress xanthine oxidase activity in dairy products: an improved HPLC analysis. *Journal of Food Composition and Analysis*, vol. 48, pp. 120-127. http://doi.org/10.1016/j.jfca.2016.03.001.

REYGAERT, W.C., 2018. Green tea catechins: their use in treating and preventing infectious diseases. *BioMed Research International*, vol. 2018, pp. 9105261. http://doi.org/10.1155/2018/9105261. PMid:30105263.

SALEM, M.A., JÜPPNER, J., BAJDZIENKO, K. and GIAVALISCO, P., 2016. Protocol: a fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. *Plant Methods*, vol. 12, no. 1, 45. http://doi.org/10.1186/s13007-016-0146-2. PMid:27833650.

SAKLAR, S., ERTAS, E., OZDEMIR, I.S. and KARADENIZ, B., 2015. Effects of different brewing conditions on catechin content and sensory acceptance in Turkish green tea infusions. *Journal of Food Science and Technology*, vol. 52, no. 10, pp. 6639-6646. http://doi.org/10.1007/s13197-015-1746-y. PMid:26396411.

TCHAMGOUE, J., NGANDJUI, Y.A.T., TALLA, R.M., AMBAMBA, B.D.A., TCHOUANKEU, J.C. and KOUAM, S.F., 2023. Extraction of phytoconstituents for lifestyle diseases. In: A.K. DHARA, S.C. MANDAL, eds. *Role of herbal medicines: management of lifestyle diseases*. Singapore: Springer, pp. 33-58. http://doi.org/10.1007/978-981-99-7703-1_3.

WANG, R., ZHOU, W. and JIANG, X., 2008. Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range. *Journal of Agricultural and Food Chemistry*, vol. 56, no. 8, pp. 2694-2701. http://doi.org/10.1021/jf0730338. PMid:18361498.

YANG, C.S. and LANDAU, J.M., 2000. Effects of tea consumption on nutrition and health. *The Journal of Nutrition*, vol. 130, no. 10, pp. 2409-2412. http://doi.org/10.1093/jn/130.10.2409. PMid:11015465.

Supplementary Material

Supplementary material accompanies this paper.

Supplementary Material S1. Fit Model.

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