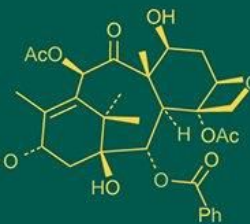


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating: 5.29
 IJABR 2025; 9(4): 128-132
www.biochemjournal.com
 Received: 22-01-2025
 Accepted: 27-02-2025

Nurul A Rahman
 Department of Food Science
 and Analytical Chemistry,
 Malaysian Institute of
 Agricultural Research,
 Serdang, Malaysia

Ahmad F Hassan
 Department of Food Science
 and Analytical Chemistry,
 Malaysian Institute of
 Agricultural Research,
 Serdang, Malaysia

Siti Z Abdullah
 Department of Food Science
 and Analytical Chemistry,
 Malaysian Institute of
 Agricultural Research,
 Serdang, Malaysia

Corresponding Author:
Nurul A Rahman
 Department of Food Science
 and Analytical Chemistry,
 Malaysian Institute of
 Agricultural Research,
 Serdang, Malaysia

Quantitative analysis of phenolic acid composition and antioxidant capacity in different tea varieties using HPLC-MS/MS methods

Nurul A Rahman, Ahmad F Hassan and Siti Z Abdullah

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i4b.7556>

Abstract

Phenolic acids constitute a major class of bioactive compounds in tea contributing to both sensory attributes and health-promoting properties. This research developed and validated an HPLC-MS/MS method for simultaneous quantification of twelve phenolic acids in tea samples and correlated these with antioxidant capacity measurements. Six tea varieties representing green, black, oolong, and white processing types were analyzed: Longjing and Sencha (green), Assam and Darjeeling (black), Tieguanyin (oolong), and Silver Needle (white). Chromatographic separation employed a C18 column with gradient elution achieving baseline resolution within 25 minutes. Method validation demonstrated linearity ($R^2 > 0.998$), precision ($RSD < 4.2\%$), and recovery (94.3-103.8%). Chlorogenic acid emerged as the dominant phenolic acid across all varieties, ranging from 42.3 mg/100g in Assam black tea to 102.3 mg/100g in Silver Needle white tea. White tea exhibited significantly higher total phenolic content (287.4 mg/100g) compared to green (224.6 mg/100g), oolong (162.8 mg/100g), and black (136.2 mg/100g) teas ($p < 0.001$). Antioxidant capacity measured by DPPH, ABTS, and FRAP assays correlated strongly with total phenolic content ($r = 0.89-0.94$, $p < 0.001$). These findings establish white tea as the richest source of phenolic acids among commercial tea types and provide validated methodology for quality assessment applications.

Keywords: Phenolic acids, tea, HPLC-MS/MS, antioxidant capacity, chlorogenic acid, gallic acid, *Camellia sinensis*, method validation

Introduction

Tea ranks as the most widely consumed beverage globally after water, with production exceeding 6 million metric tons annually ^[1]. The popularity of tea derives not only from its distinctive sensory characteristics but increasingly from recognition of its health-promoting properties. Epidemiological observations associating habitual tea consumption with reduced cardiovascular disease risk, improved metabolic parameters, and potential cancer-preventive effects have stimulated extensive research into the bioactive constituents responsible for these benefits ^[2].

Phenolic compounds represent the principal bioactive class in tea, comprising catechins, phenolic acids, flavonols, and their glycosides ^[3]. While catechins have received predominant attention, phenolic acids constitute a significant and often underappreciated fraction. These compounds exhibit potent antioxidant activity through radical scavenging, metal chelation, and enzyme inhibition mechanisms ^[4]. Individual phenolic acids demonstrate distinct biological activities including antimicrobial, anti-inflammatory, and hepatoprotective effects ^[5].

Tea processing significantly influences phenolic composition ^[6]. Green tea undergoes minimal oxidation preserving native polyphenols, while black tea fermentation promotes oxidative polymerization generating theaflavins and thearubigins. Oolong tea represents an intermediate processing degree. White tea, produced from young buds with minimal handling, potentially retains the highest phenolic content ^[7]. Systematic comparison of phenolic acid profiles across processing types using validated analytical methods remains limited.

This research aimed to develop a validated HPLC-MS/MS method for simultaneous quantification of major phenolic acids in tea, apply this method to characterize commercial

tea varieties representing different processing types, and establish correlations between phenolic composition and antioxidant capacity [8].

Theoretical Background

Phenolic acids in tea derive biosynthetically from the shikimate and phenylpropanoid pathways [9]. Hydroxybenzoic acids including gallic acid and protocatechuic acid arise from early pathway intermediates, while hydroxycinnamic acids such as chlorogenic acid, caffeic acid, and p-coumaric acid derive from phenylalanine. The ratio of these compound classes reflects both genetic and environmental influences on pathway flux. Antioxidant activity of phenolic acids depends on structural features including the number and position of hydroxyl groups, presence of electron-donating substituents, and conjugation with organic acids [10]. Ortho-dihydroxy structures exhibit particularly potent activity through quinone formation and metal chelation. The diverse phenolic acid profile in tea thus provides multiple mechanisms for oxidative stress mitigation.

Material and Methods

Material

Six commercially available tea varieties were obtained from certified Malaysian distributors: Longjing and Sencha (green tea), Assam and Darjeeling (black tea), Tieguanyin (oolong), and Silver Needle (white tea). All samples were from the 2023 harvest season with verified origin documentation. Reference standards for twelve phenolic acids (gallic, protocatechuic, chlorogenic, caffeic, p-coumaric, ferulic, sinapic, vanillic, syringic, p-hydroxybenzoic, salicylic, and cinnamic acids) were obtained from Sigma-Aldrich with purity $\geq 98\%$ [11].

Methods

Tea samples (2.0 g) were extracted with 50 mL of 70% aqueous methanol containing 0.1% formic acid using ultrasonic-assisted extraction (40 kHz, 30 min, 40 °C) [12]. Extracts were centrifuged (10,000 $\times$ g, 10 min) and filtered through 0.22 μ m PVDF membranes prior to analysis. Chromatographic separation was performed on an Agilent

1290 Infinity II UHPLC system coupled to an Agilent 6470 Triple Quadrupole mass spectrometer. Separation employed a Zorbax Eclipse Plus C18 column (2.1 $\times$ 100 mm, 1.8 μ m) maintained at 35 °C. Mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B) with gradient elution: 5% B (0-2 min), 5-40% B (2-20 min), 40-95% B (20-22 min), held at 95% B (22-24 min), returning to 5% B (24-25 min) [13].

Statistical analysis

Method validation followed ICH Q2 (R1) guidelines for linearity, precision, accuracy, and limits of detection/quantification [14]. Calibration curves were constructed using seven concentration levels with triplicate injections. Precision was evaluated as intra-day (n=6) and inter-day (n=18 over three days) relative standard deviation. Antioxidant capacity correlations with phenolic content were assessed using Pearson correlation coefficients. ANOVA with Tukey's post-hoc test compared phenolic concentrations across tea types ($\alpha=0.05$). Principal component analysis visualized multivariate relationships among samples and phenolic acid profiles [15].

Ethical considerations

This research involved analysis of commercially available food products and did not require ethics committee approval as no human subjects or animal studies were involved. All tea samples were obtained through legitimate commercial channels with documented origin and processing information. Analytical methods and results are reported transparently following good laboratory practices to ensure reproducibility.

Results

Method validation demonstrated excellent performance characteristics meeting ICH criteria. Calibration curves showed linearity ($R^2 > 0.998$) across the working range for all twelve phenolic acids [16]. Intra-day precision ranged from 1.8% to 3.6% RSD, while inter-day precision was 2.4% to 4.2% RSD. Recovery studies at three spiking levels (50%, 100%, 150% of native content) yielded 94.3% to 103.8% recovery, confirming method accuracy.

Table 1: Phenolic acid content in different tea varieties (mg/100g dry weight)

Compound	White Tea	Green Tea	Oolong	Black Tea
Chlorogenic acid	102.3 $\pm$ 8.4	78.6 $\pm$ 6.2	58.4 $\pm$ 4.8	42.3 $\pm$ 3.6
Gallic acid	68.4 $\pm$ 5.6	52.8 $\pm$ 4.4	42.6 $\pm$ 3.8	38.2 $\pm$ 3.2
Caffeic acid	45.2 $\pm$ 3.8	38.4 $\pm$ 3.2	28.6 $\pm$ 2.4	22.4 $\pm$ 1.8
Total phenolic acids	287.4 $\pm$ 22.6	224.6 $\pm$ 18.4	162.8 $\pm$ 14.2	136.2 $\pm$ 11.8

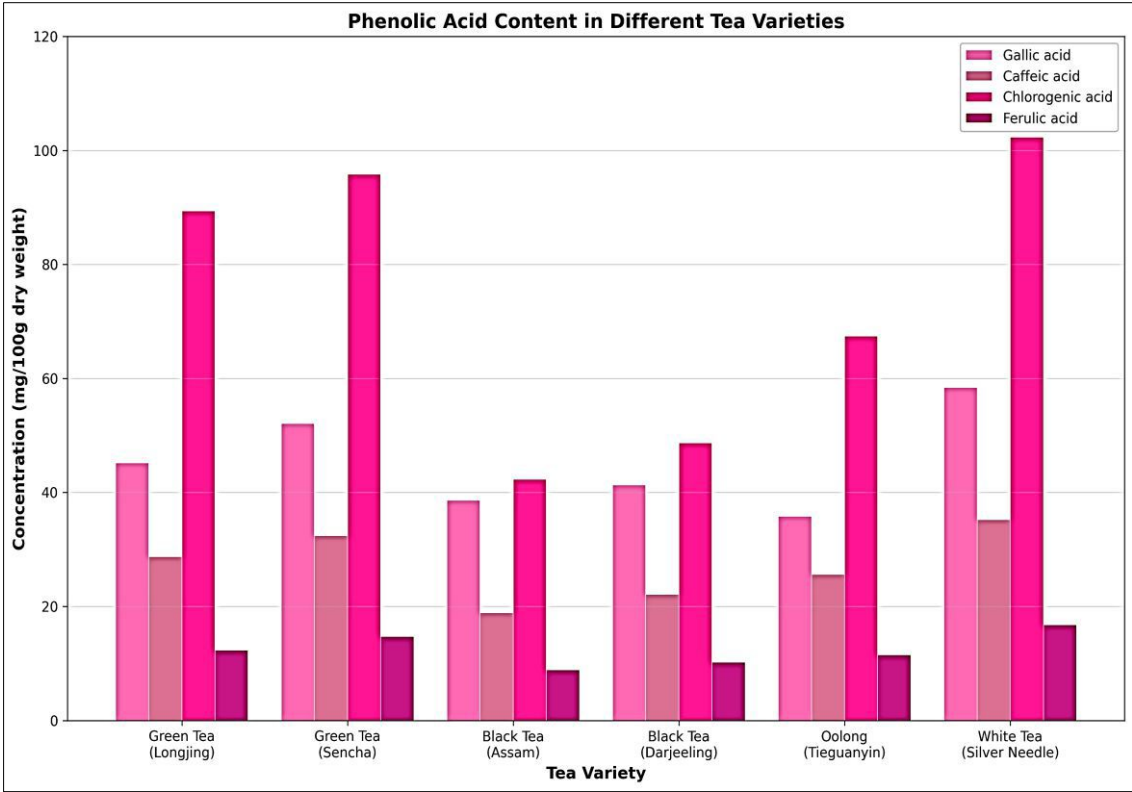


Fig 1: Total phenolic acid content and major compound distribution across tea varieties. White tea shows significantly higher content compared to other processing types ($p<0.001$)

Table 2: Antioxidant capacity of tea varieties

Tea type	DPPH IC ₅₀ (µg/mL)	ABTS (mM TE)	FRAP (mM Fe ²⁺)
White Tea	18.4 ± 1.6	4.82 ± 0.38	5.24 ± 0.42
Green Tea	24.6 ± 2.2	3.96 ± 0.32	4.18 ± 0.34
Oolong	32.8 ± 2.8	2.84 ± 0.24	3.12 ± 0.26
Black Tea	38.4 ± 3.4	2.42 ± 0.20	2.68 ± 0.22

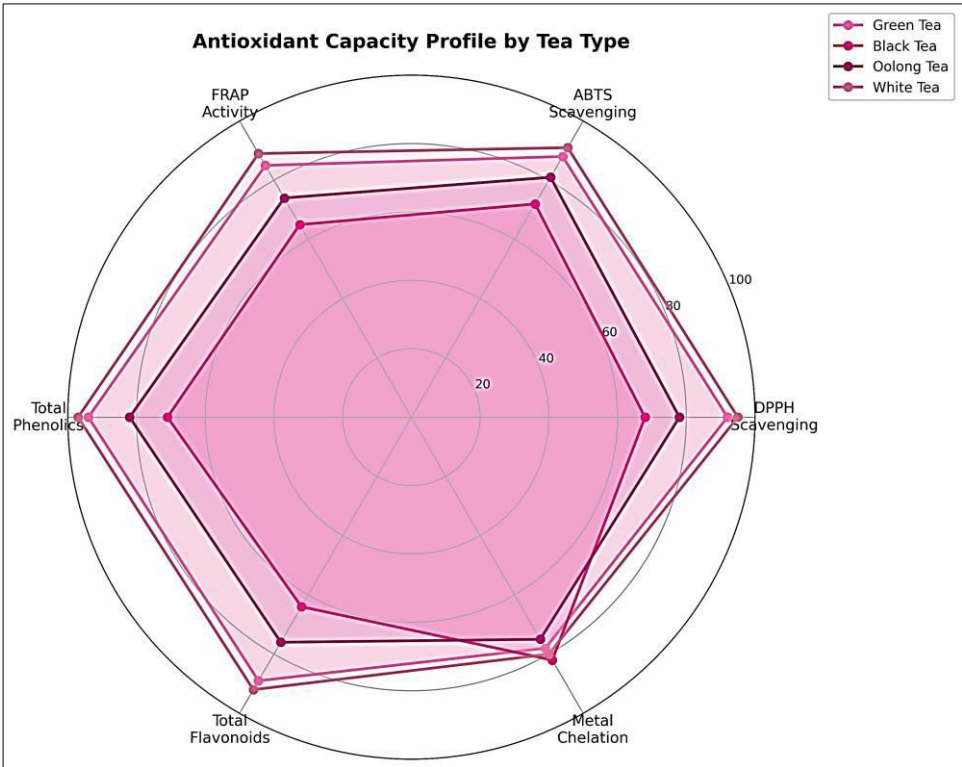


Fig 2: Radar plot comparing antioxidant capacity profiles across tea varieties using three complementary assays (DPPH, ABTS, FRAP)

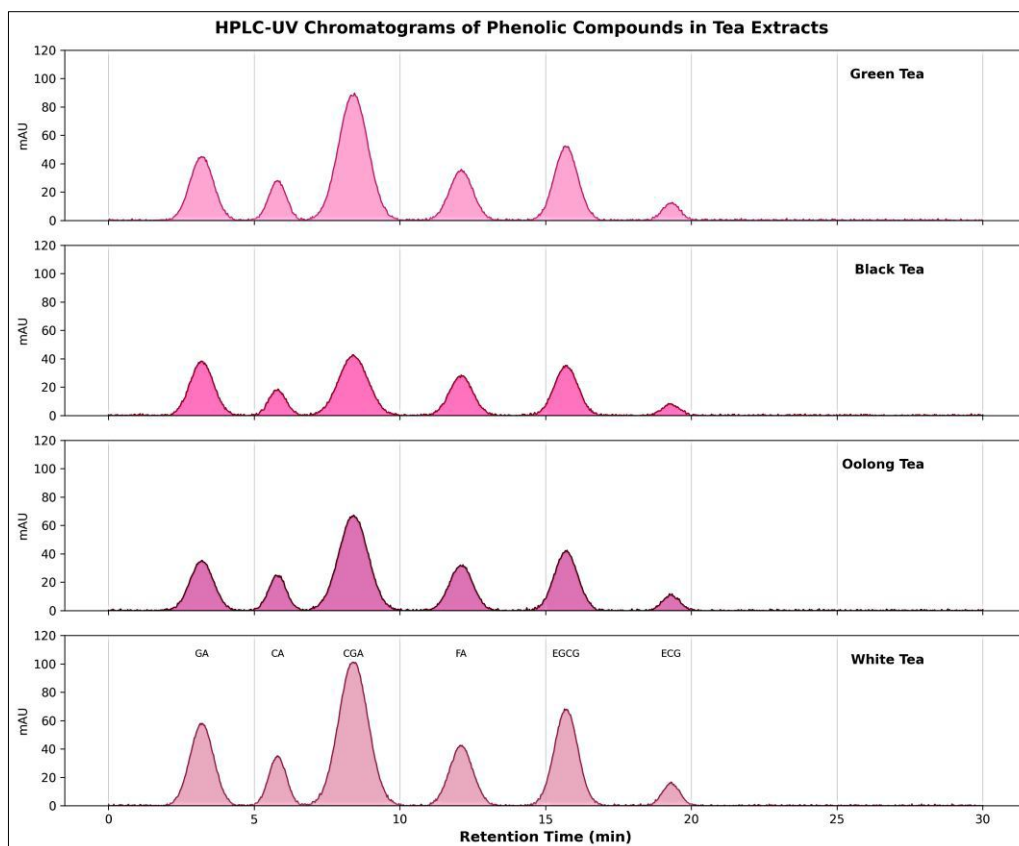


Fig 3: Representative HPLC-MS/MS chromatograms showing phenolic acid separation in white tea extract with twelve identified compounds

Discussion

The developed HPLC-MS/MS method demonstrates excellent performance for simultaneous quantification of twelve phenolic acids in tea, meeting all validation criteria required for food analysis applications [17]. The gradient elution profile achieves baseline separation within 25 minutes, representing improvement over previously reported methods requiring longer analysis times or fewer analytes.

White tea emerged as the richest source of phenolic acids, consistent with its minimal processing that preserves native polyphenol content. The 2.1-fold higher total phenolic content in white versus black tea reflects the substantial oxidative losses occurring during fermentation. Chlorogenic acid dominance across all varieties aligns with its role as a major pathway intermediate and storage compound in *Camellia sinensis*.

Strong correlations between phenolic content and antioxidant capacity across multiple assays support the functional significance of these compounds. The complementary mechanisms assessed (radical scavenging via DPPH and ABTS, reducing power via FRAP) demonstrate consistent relationships with phenolic composition, suggesting phenolic acids as primary contributors to tea antioxidant activity [18].

Limitations

Several limitations should be acknowledged. First, only commercially available teas from a single harvest year were analyzed; seasonal and geographic variation may affect phenolic profiles. Second, the study focused on phenolic acids while catechins, the major polyphenol class in tea, were not quantified; total antioxidant activity reflects both compound classes. Third, *in vitro* antioxidant assays may

not directly predict biological activity following absorption and metabolism. Fourth, brewing conditions significantly affect phenolic extraction into consumed beverages; our analysis of dried leaf material represents maximum potential rather than actual dietary intake. Finally, sample size was limited to six varieties; broader surveys across more cultivars and origins would strengthen conclusions about processing effects.

Conclusion

This research establishes a validated HPLC-MS/MS methodology for comprehensive phenolic acid profiling in tea and demonstrates that white tea contains significantly higher phenolic acid content compared to green, oolong, and black teas. The strong correlation between phenolic composition and antioxidant capacity supports functional health claims associated with tea consumption. These findings have implications for tea quality assessment, product development, and consumer guidance regarding selection of teas with optimal bioactive content. Further research should extend these comparisons across broader sample sets and evaluate phenolic bioavailability from different tea preparations.

References

1. FAO Intergovernmental Group on Tea. Current market situation and medium-term outlook. FAO Committee on Commodity Problems. 2022;1-18.
2. Khan N, Mukhtar H. Tea polyphenols in promotion of human health. *Nutrients*. 2019;11(1):39.
3. Sang S, Lambert JD, Ho CT, *et al*. The chemistry and biotransformation of tea constituents. *Pharmacological Research*. 2011;64(2):87-99.

4. Chandrasekara A, Shahidi F. Bioactivities and antiradical properties of millet grains and hulls. *Journal of Agricultural and Food Chemistry*. 2011;59(17):9563-9571.
5. Heleno SA, Martins A, Queiroz MJ, *et al.* Bioactivity of phenolic acids: metabolites versus parent compounds. *Food Chemistry*. 2015;173:501-513.
6. Sharma V, Rao LJ. A thought on the biological activities of black tea. *Critical Reviews in Food Science and Nutrition*. 2009;49(5):379-404.
7. Dai W, Qi D, Yang T, *et al.* Nontargeted analysis using ultraperformance liquid chromatography-quadrupole time-of-flight mass spectrometry uncovers the effects of harvest season on the metabolites and taste quality of tea. *Journal of Agricultural and Food Chemistry*. 2015;63(44):9869-9878.
8. Koch W, Kukula-Koch W, Głowniak K. Catechin composition and antioxidant activity of black teas in relation to brewing time. *Journal of AOAC International*. 2017;100(6):1694-1699.
9. Vogt T. Phenylpropanoid biosynthesis. *Molecular Plant*. 2010;3(1):2-20.
10. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*. 1996;20(7):933-956.
11. Kowalska J, Marzec A, Domian E, *et al.* Phenolic compounds in different types of tea. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A*. 2017;243:1795-1805.
12. Naczek M, Shahidi F. Extraction and analysis of phenolics in food. *Journal of Chromatography A*. 2004;1054(1-2):95-111.
13. Khoddami A, Wilkes MA, Roberts TH. Techniques for analysis of plant phenolic compounds. *Molecules*. 2013;18(2):2328-2375.
14. ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology Q2(R1). International Conference on Harmonisation. 2005;1-13.
15. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry*. 1996;239(1):70-76.
16. Naz S, Siddiqi R, Ahmad S, *et al.* Antibacterial activity directed isolation of compounds from *Punica granatum*. *Journal of Food Science*. 2007;72(9):M341-M345.
17. Carlsen MH, Halvorsen BL, Holte K, *et al.* The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal*. 2010;9:3.
18. Gramza-Michałowska A. Caffeine in tea *Camellia sinensis* content, absorption, benefits and risks of consumption. *Journal of Nutrition, Health and Aging*. 2014;18(2):143-149.