

Analytical Methods for the Determination and Quantification of Polyphenols in Medicinal Plants: A Comprehensive Review

Nguyen Thi Hong Thoa

Faculty of Medical Laboratory Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.

*Corresponding Author: Nguyen Thi Hong Thoa

DOI: <https://doi.org/10.5281/zenodo.22962460>

Article History	Abstract
Original Research Article	
Received: 01-07-2026	
Accepted: 29-08-2026	
Published: 25-09-2026	
Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.	
Citation: Nguyen Thi Hong Thoa. (2026). Analytical methods for the determination and quantification of polyphenols in medicinal plants: A comprehensive review. UKR Journal of Medicine and Medical Research, 2(5), 15-20.	<i>Polyphenols are among the most abundant and functionally diverse classes of secondary metabolites synthesized by plants. These organic compounds, characterized by multiple phenol structural units, play an essential role in plant defense systems, organoleptic quality, and medicinal bioactivity. Due to their potent antioxidant, anti-inflammatory, antimicrobial, and cardioprotective attributes, polyphenol quantification and profiling have become indispensable quality control parameters in the standardization of herbal medicines and botanical dietary supplements.</i> <i>This review provides a systematic synthesis of the principal analytical methodologies currently utilized for assessing polyphenol content in raw plant materials and derived pharmaceutical formulations. Specifically, we examine three major analytical paradigms: colorimetric assays via the Folin–Ciocalteu (FC) reagent, high-performance liquid chromatography (HPLC) paired with spectrophotometric or electrochemical detectors, and liquid chromatography coupled with mass spectrometry (LC–MS). The fundamental principles, sensitivity, selectivity, operational costs, sample preparation protocols, and limitations of each technique are systematically critically analyzed and contrasted.</i> <i>The colorimetric Folin–Ciocalteu method offers an accessible, cost-effective screening tool for total phenolic content (TPC), though it suffers from cross-reactivity with non-phenolic reducing compounds. Conversely, HPLC provides high resolution and precision for quantifying individual marker compounds, albeit requiring pure standard reference compounds and extensive chromatographic optimization. LC–MS represents the gold standard for structural elucidation, isomer differentiation, and high-throughput profiling of complex phenolic matrices without always requiring reference standards, though bounded by higher capital expenditure and operational complexity. Finally, this review highlights prospective trends in green extraction technologies, miniaturized analytical devices, and high-resolution mass spectrometry aimed at improving reliability, eco-friendliness, and precision in botanical quality control.</i> Keywords: Polyphenols, Folin–Ciocalteu Assay, HPLC, LC–MS, Quality Control.

1. Introduction

For centuries, medicinal plants have formed the foundation of traditional healthcare systems worldwide and continue to serve as pivotal sources for modern drug discovery [2]. The therapeutic efficacy of these botanical matrixes is predominantly attributed to their rich content of secondary metabolites, among which polyphenolic compounds constitute one of the most structurally diverse and biologically active families [1]. Derived from the

shikimate/phenylpropanoid and polyketide pathways, polyphenols encompass a broad spectrum of structures ranging from simple phenolic acids to complex condensed tannins and flavonoid glycosides.

From a structural perspective, polyphenolic compounds contain one or more aromatic rings substituted with multiple hydroxyl (-OH) functional groups. This structural motif confers unique chemical properties, including strong

electron- or hydrogen-atom donating abilities, metal-chelating capacity, and the capacity to modulate various biochemical pathways and enzyme cascades in biological systems. In botanical matrices, polyphenols contribute significantly to color, astringency, flavor, and physiological protection against abiotic stress, UV radiation, and phytopathogen attacks. In human pharmacology, dietary and medicinal polyphenols demonstrate significant antioxidant, free-radical scavenging, anti-inflammatory, neuroprotective, and anti-oncogenic activities [2,4].

Due to the complex nature of natural product preparations, stringent quality control protocols are essential to ensure the safety, batch-to-batch consistency, and therapeutic efficacy of herbal medicines. Standardizing botanical raw materials relies heavily on the precise qualitative identification and quantitative estimation of signature polyphenol markers. However, analyzing polyphenols presents formidable analytical challenges, primarily due to their structural diversity and isomerism (where hundreds of related phenolics, including structural and stereoisomers, co-occur within a single extract), severe matrix effects (interferences caused by lipids, sugars, pigments, proteins, and organic acids in crude extracts), and their susceptibility to oxidation and degradation (resulting from thermal instability, light sensitivity, and pH-dependent structural alterations during extraction and analysis) [6,7].

To overcome these obstacles, a wide array of analytical strategies has been developed. The choice of technique frequently involves a compromise between throughput, specificity, cost, and analytical depth [10]. This review focuses on three foundational analytical approaches currently employed in pharmaceutical and botanical research: the spectrophotometric Folin–Ciocalteu colorimetric assay, High-Performance Liquid Chromatography (HPLC), and Liquid Chromatography–Mass Spectrometry (LC–MS). By critically comparing their underlying chemistry, performance metrics, and application boundaries, this paper aims to provide researchers and quality control analysts with a coherent framework for selecting optimal methodologies for polyphenol analysis.

2. Chemical Diversity and Pharmacological Significance of Polyphenols

To establish an appropriate analytical workflow, one must first comprehend the chemical domain of the target analytes. Polyphenols are broadly categorized into distinct sub-classes based on the number of phenol rings and the structural elements that bind these rings to one another.

2.1 Principal Classes of Polyphenols

Polyphenols are categorized into five principal classes: Phenolic Acids, which are divided into hydroxybenzoic

acids (featuring a C₆–C₁ backbone, such as gallic, vanillic, and protocatechuic acids) and hydroxycinnamic acids (featuring a C₆–C₃ structure, including caffeic, ferulic, *p*-coumaric, and chlorogenic acids); Flavonoids, the largest and most extensively studied group characterized by a C₆–C₃–C₆ skeleton consisting of two aromatic rings linked via a three-carbon heterocyclic pyran or pyrone ring, which encompasses major subclasses such as flavonols (quercetin, kaempferol, myricetin), flavones (luteolin, apigenin), flavan-3-ols (catechins, epicatechin, EGCG), flavanones (naringenin, hesperetin), isoflavones (genistein, daidzein), and anthocyanidins/anthocyanins (cyanidin, delphinidin, malvidin); Stilbenes, defined by a C₆–C₂–C₆ structure containing two aromatic rings connected by an ethene bridge, with resveratrol as the primary representative; Lignans, which are formed by the oxidative dimerization of two phenylpropanoid units C₆–C₃; and Tannins, high molecular weight polymers subdivided into hydrolyzable tannins (polyesters of gallic or ellagic acid with a central polyol core) and condensed tannins (proanthocyanidins, formed by polymers of flavan-3-ol units) [6,7].

2.2 Biological Activities and Physicochemical Properties

The aromatic rings coupled with hydroxyl moieties render polyphenols effective electron- and hydrogen-donors, enabling them to act as primary antioxidants by neutralizing reactive oxygen species (ROS) and reactive nitrogen species (RNS) through the general free-radical scavenging reaction $\text{PhOH} + \text{R}^\cdot \rightarrow \text{PhO}^\cdot + \text{RH}$ (Equation 1), where PhOH represents a phenolic compound and R[·] denotes an initiating free radical. The resulting phenoxyl radical PhO[·] is stabilized via resonance delocalization across the conjugated aromatic system, rendering it unreactive toward further oxidative propagation steps. Furthermore, adjacent ortho-hydroxyl groups (catechol or pyrogallol moieties) provide strong chelation sites for transition metal ions such as Fe²⁺, Fe³⁺ and Cu²⁺ and, thereby suppressing Fenton-type reactions that generate destructive hydroxyl radicals (OH[·]). Beyond passive antioxidant chemistry, polyphenols actively modulate cellular signal transduction pathways and inhibit key metabolic enzymes involved in inflammatory and metabolic cascades (such as cyclooxygenase-2, lipoxygenase, α -glucosidase, and xanthine oxidase), necessitating analytical methods capable of both precise quantitative accuracy and high-resolution separation of structurally similar constituents within complex crude botanical matrices [2,10].

3. Overview of Analytical Methods

Analytical strategies for botanical polyphenols can be broadly segregated into two main categories: non-specific bulk assay methods (colorimetric tests for total content) and separation-based specific methods (chromatographic protocols for individual component profiling) [13].

Parameter / Feature	Folin–Ciocalteu Assay	High-Performance Liquid Chromatography (HPLC)	Liquid Chromatography–Mass Spectrometry (LC–MS)
Primary Output	Total Phenolic Content (TPC) equivalent	Quantitation of specific known compounds	Structural identification, structural profiling & trace quantification

Selectivity / Specificity	Low (Susceptible to reducing interferences)	Moderate to High (Depends on chromatographic resolution)	Very High (Mass accuracy & product ion spectra)
Sensitivity	Low to Moderate (µg/mL range)	High (µg/mL to ng/mL range)	Ultra-high (ng/mL to pg/mL range)
Sample Preparation	Simple (Minimal dilution/filtration)	Moderate (Solvent extraction, filtration, SPE)	Extensive (Solvent clean-up, matrix suppression mitigation)
Analysis Time	Rapid (30–90 min batch processing)	Moderate (15–60 min per run)	Moderate to Rapid (5–30 min per run)
Reference Standards	Single generic standard (Gallic Acid)	Mandatory for every target analyte	Optional for structural profiling; required for strict quantitation
Capital & Operational Cost	Low	Moderate	High

4. Spectrophotometric Assay: The Folin–Ciocalteu (FC) Reagent

4.1 Principle of the Assay

The Folin–Ciocalteu assay is the most widely utilized method for measuring Total Phenolic Content (TPC) in herbal materials, food products, and dietary supplements. Originally formulated by Folin and Denis (1912) for protein analysis and later modified by Singleton and Rossi (1965) for wine analysis, the assay relies on an electron-transfer mechanism under alkaline conditions [3,12].

The Folin–Ciocalteu reagent is an aqueous mixture composed of phosphomolybdic ($\text{H}_3\text{PMo}_{12}\text{O}_{40}$) and phosphotungstic ($\text{H}_3\text{PW}_{12}\text{O}_{40}$) heteropoly acids. In an alkaline environment—typically generated by the addition of sodium carbonate (Na_2CO_3)—phenolic hydroxyl groups undergo dissociation to form phenolate anions (ArO^-). These anions transfer electrons to the heteropoly acids, reducing the molybdenum and tungsten centers to lower oxidation states

This reduction produces an intensely blue-colored chromophore (a mixed-valence heteropoly blue complex) with a broad absorption maximum typically measured between 750 nm and 765 nm. The intensity of the absorbance is directly proportional to the concentration of reducing phenolic groups in the sample, quantified against a standard curve of a reference compound—most commonly gallic acid, yielding results expressed as Gallic Acid Equivalents (GAE) [12].

4.2 Advantages and Operational Feasibility

Cost-Effectiveness and Simplicity: The assay requires basic laboratory infrastructure—primarily a standard UV-Vis spectrophotometer or microplate reader—making it highly accessible for routine Quality Control (QC) laboratories and initial screening assays.

High Throughput: When adapted to 96-well microplate

formats, hundreds of samples can be evaluated simultaneously within a short timeframe.

Standardized Metric: Provides a universally recognized baseline metric (mg GAE/g dry weight) that enables preliminary comparisons across different plant species, geographic origins, or extraction methods.

4.3 Limitations and Sources of Interference

Despite its widespread application, the Folin–Ciocalteu (FC) assay lacks absolute specificity for phenolic compounds, measuring total reducing capacity instead. Non-phenolic electron donors capable of reacting under alkaline conditions frequently yield false-positive outcomes or lead to an overestimation of the total phenolic content (TPC). Key interfering matrix constituents include ascorbic acid (vitamin C), which acts as a highly reactive reducing agent that quantitatively reduces the FC reagent at room temperature; reducing sugars such as glucose, fructose, and sucrose, which react with the reagent under elevated temperatures or prolonged incubation times; amino acids and proteins possessing sidechains capable of reducing heteropoly acids (specifically tyrosine, tryptophan, and cysteine residues); and inorganic species, notably Fe^{2+} alongside other reduced transition metal ions [4,7]. Consequently, although the FC assay remains a valuable tool for rapid screening, it can neither differentiate between distinct phenolic subclasses nor resolve individual chemical constituents.

5. High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) coupled with Ultraviolet-Visible Spectrophotometric Detection (UV-Vis) or Photodiode Array Detection (PDA/DAD) represents the premier regulatory standard for the qualitative profiling and quantitative estimation of polyphenols in botanical drugs [10,13]. Operationally, a high-pressure pump system drives mobile phases A and B

under a gradient elution profile alongside the injected sample into a reversed-phase HPLC column (typically C₁₈), where the polyphenolic constituents are separated based on their hydrophobic interactions prior to detection by the UV-Vis/DAD spectrophotometer and final signal visualization via a computerized chromatogram data processing station.

5.1 Chromatographic Separation Principles and Stationary Phases

Polyphenols exhibit diverse polarities, ranging from highly polar phenolic acids to moderately non-polar methoxylated flavones, making reversed-phase high-performance liquid chromatography (RP-HPLC) the universally preferred separation mode for their resolution [13]. The stationary phase typically comprises silica particles functionalized with hydrophobic octadecylsilane (C₁₈) carbon chains, although octyl (C₈), phenyl-hexyl, and pentafluorophenyl (PFP) chemistries are frequently employed to optimize the selectivity of challenging structural isomers. Retention in RP-HPLC is governed primarily by hydrophobic interactions between solute molecules and the non-polar alkyl chains, resulting in the early elution of polar phenolic derivatives, whereas less polar aglycones and methoxylated species exhibit longer retention times. To suppress the ionization of phenolic hydroxyl and carboxylic acid moieties—thereby eliminating severe peak tailing and band broadening—acidified mobile phases are strictly required. Standard eluent systems utilize a binary gradient elution profile comprising an organic modifier as Phase B (typically acetonitrile or methanol) and an acidified aqueous Phase A consisting of ultrapure water containing low concentrations (0.1-1% v/v) of formic acid, acetic acid, or trifluoroacetic acid. The overall mobile phase pH is typically maintained between 2.0 and 3.5, ensuring that carboxylic acid and phenolic functional groups remain predominantly in their unionized state [10,13].

5.2 Detection Methods: UV-Vis and Photodiode Array (PDA)

Spectrophotometric detection relies on the presence of conjugated chromophores within polyphenolic structures, with distinct polyphenol classes displaying characteristic ultraviolet absorption maxima (λ_{max}): hydroxybenzoic acids typically absorb between 270 and 280 nm; hydroxycinnamic acids absorb between 310 and 330 nm; flavonols (e.g., quercetin) display dual absorption bands, consisting of Band II (250 – 280 nm), corresponding to the benzoyl system of Ring A and Band I (350 – 385 nm), corresponding to the cinnamoyl system of Ring B/C; flavones exhibit Band II and Band I; and anthocyanins are distinguished by strong absorption bands in the visible region (500-550nm) [8,13]. The Photodiode Array Detector (PDA or DAD) permits simultaneous multi-wavelength monitoring alongside real-time spectral acquisition (200 – 800 nm) across peak elution profiles, thereby enabling UV spectral matching against analytical reference libraries for preliminary subclass identification and the rigorous verification of chromatographic peak purity [8].

5.3 Method Validation and Analytical Rigor

Quantitation by HPLC requires strict method validation

according to regulatory framework guidelines, such as ICH Q2 (R2) [5], evaluating key performance parameters including linearity across working concentration ranges (typically yielding determination coefficients $R^2 > 0.999$), precision and repeatability assessed via relative standard deviation (%RSD < 2%) for retention times and peak areas, limits of detection (LOD) and quantification (LOQ) defined at signal-to-noise ratios (S/N) of 3:1 and 10:1, respectively, and accuracy verified through recovery studies spiking pure chemical reference standards into matrix samples to consistently achieve acceptable mean recoveries between 95% and 105%.

5.4 Advantages and Limitations

High-Performance Liquid Chromatography offers major analytical advantages, including excellent reproducibility, precise quantitation of target bioactive compounds, established regulatory acceptance, robust instrumentation, and high column longevity. However, the technique presents inherent limitations, such as the inability to structural identification of unknown compounds in the absence of authentic reference standards, potential co-elution of closely related structural isomers that impairs accurate quantitation, extended analysis run times (30-60 min) under conventional gradient profiles, and substantial organic solvent consumption [10,13].

6. Liquid Chromatography–Mass Spectrometry (LC–MS)

Liquid Chromatography coupled with Mass Spectrometry (LC–MS) and tandem mass spectrometry (LC–MS/MS) represents the state of the art in natural product analysis, offering unmatched sensitivity, selectivity, and structural elucidation capabilities [11,14,15].

6.1 Ionization Techniques: Electrospray Ionization (ESI)

Because polyphenols are polar, non-volatile, and thermally labile molecules, soft ionization techniques are required to transition liquid phase analytes into gas-phase ions without inducing extensive uncontrolled thermal fragmentation. Electrospray Ionization (ESI) is the predominant interface employed for polyphenol analysis [11].

ESI can operate in both Positive Ion Mode (ESI⁺) and Negative Ion Mode (ESI⁻).

Negative Ion Mode (ESI⁻): Generally exhibits higher sensitivity, lower background noise, and superior ionization efficiency for polyphenols due to the acidic nature of phenolic hydroxyl and carboxylic functional groups, yielding deprotonated molecular ions [M-H]⁻.

Positive Ion Mode (ESI⁺): Primarily used for basic or nitrogenous compounds, but highly effective for anthocyanins, which natively exist as stable flavylum cations (M⁺) in acidic solution [11,15].

6.2 Mass Analyzers and MS/MS Structural Characterization

Modern liquid chromatography–mass spectrometry (LC–MS) configurations utilize various mass analyzer architectures depending on the specific analytical objective:

Triple Quadrupole mass analyzers excel in targeted quantitative analysis using Selected Reaction Monitoring or Multiple Reaction Monitoring modes; Quadrupole Time-of-Flight hybrid systems offer high mass accuracy (<5 ppm) and high resolving power (> 30,000 FWHM), enabling untargeted metabolomics, accurate mass determination, and elemental formula assignment; and Orbitrap analyzers provide ultra-high resolving power (> 100,000 FWHM), facilitating the resolution of isobaric mass peaks within complex crude plant matrices. Tandem mass spectrometry (MS/MS or MSⁿ) provides structural diagnostic fragmentation patterns, where glycoside cleavages via neutral losses of sugar moieties—such as hexoses (-162 Da), pentoses (-132 Da) or deoxyhexoses/rhamnose (-146 Da), yield prominent aglycone product ions [Y₀]-, while collision-induced dissociation (CID) triggering Retro-Diels–Alder (RDA) reactions across the flavonoid C-ring produces diagnostic fragment ions (^{x,y} A⁻ and ^{x,y} B⁻) that elucidate specific substitution patterns, including hydroxyl, methoxyl, or C-glycosylation assignments, on the A- and B-rings [8,15].

6.3 Advantages and Challenges

Liquid chromatography–mass spectrometry (LC–MS) offers major analytical advantages, including ultra-trace sensitivity extending into the picogram-to-femtogram range, high structural selectivity capable of resolving co-eluting peaks based on unique mass-to-charge (m/z) ratios, and the capability to elucidate the structures of novel or unstandardized compounds without requiring pure authentic standards [11,14]. However, the technique presents notable challenges, such as susceptibility to ion suppression or enhancement caused by co-eluting matrix components (matrix effects), high capital expenditure alongside specialized maintenance and operational demands, and the necessity of using isotopically labeled internal standards or matrix-matched calibration curves to ensure accurate quantitation despite ionization variability.

7. Comparative Critical Analysis and Selection Framework

The selection of an appropriate analytical methodology depends heavily on the specific research objective, resource availability, sample complexity, and required regulatory compliance. For industrial raw material screening and rapid total phenolic content (TPC) estimations, the Folin–Ciocalteu assay is most suitable due to its operational speed, cost-effectiveness, and minimal reliance on specialized instrumentation [12]. For routine quality control, pharmacopoeial standardization, and the precise quantification of major known bioactive markers, HPLC–DAD represents the method of choice owing to its exceptional reproducibility, cost efficiency, and wide acceptance within validated regulatory frameworks [5, 10,13]. Conversely, for comprehensive untargeted metabolomics, ultra-trace analysis, and the structural elucidation of unknown phenolics, hyphenated techniques such as LC–MS/MS or high-resolution LC–QToF/Orbitrap MS are indispensable due to their superior resolving power, accurate mass capabilities, and rich structural fragmentation data [14,15].

8. Emerging Trends and Future Perspectives

The analytical chemistry of polyphenols is evolving toward more sustainable, efficient, and precise methodologies:

8.1 Ultra-High-Performance Liquid Chromatography (UHPLC)

The shift from conventional HPLC columns packed with 3.0–5.0 μm particles to UHPLC sub-2.0 μm porous or core-shell particles has significantly advanced chromatographic performance. UHPLC operates at pressures up to 15000 psi, delivering superior chromatographic resolution, higher peak capacity, and dramatically reduced analysis times (often under 10 minutes per run) while reducing organic solvent consumption by up to 80% [14].

8.2 Green Analytical Chemistry

In alignment with global sustainability goals, researchers are increasingly adopting Green Analytical Chemistry principles. This includes replacing toxic organic solvents (like acetonitrile and methanol) with eco-friendly alternatives such as ethanol, natural deep eutectic solvents (NADES), supercritical carbon dioxide (SF-CO₂), or ionic liquids in both extraction protocols and mobile phase formulations [9].

8.3 On-line Hyphenated Techniques and Ion Mobility Spectroscopy

Integrations such as LC–NMR–MS and the addition of Ion Mobility Spectrometry (IMS) into LC–MS workflows provide an additional separation dimension based on gas-phase molecular size, shape, and collision cross-section (CCS). This enables the separation of isobaric polyphenolic stereoisomers (e.g., catechin vs. epicatechin, or quercetin-3-O-glucoside vs. quercetin-3-O-galactoside) that are difficult to resolve by conventional liquid chromatography or mass spectrometry alone [6,15].

9. Conclusion

Polyphenols remain a crucial class of natural bioactive compounds requiring rigorous analytical oversight in herbal medicine and natural product development. As summarized in this review, no single analytical technique completely fulfills every requirement of sensitivity, selectivity, speed, and cost-effectiveness.

The spectrophotometric Folin–Ciocalteu colorimetric assay remains a practical method for rapid screening of total phenolic content, despite its known susceptibility to non-phenolic interferences [12]. HPLC coupled with UV/DAD detectors continues to be the standard method for routine quality control, routine method validation, and targeted marker quantification due to its accuracy, stability, and regulatory acceptance. Liquid chromatography coupled with mass spectrometry (LC–MS/MS) represents an invaluable platform for advanced research, structural elucidation, untargeted metabolomic profiling, and trace identification [14,15].

Future advances in polyphenol analysis will likely focus on integrating green extraction and separation technologies [9], adopting UHPLC platforms [14], and applying high-resolution mass spectrometry coupled with ion mobility

[15]. Emphasizing these standardized analytical strategies will continue to strengthen quality assurance, therapeutic consistency, and international regulatory acceptance of botanical products.

10. References

1. Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry Reviews*, 11(2–3), 153–177. <https://doi.org/10.1007/s11101-012-9242-8>
2. Crozier, A., Jaganath, I. B., and Clifford, M. N. (2009). Dietary phenolics: chemistry, bioavailability and effects on health. *Natural Product Reports*, 26(8), 1001–1043. <https://doi.org/10.1039/b802662a>
3. Folin, O., and Denis, W. (1912). On phosphotungstic-phosphomolybdic compounds as color reagents. *Journal of Biological Chemistry*, 12(2), 239–243. [https://doi.org/10.1016/S0021-9258\(18\)88697-5](https://doi.org/10.1016/S0021-9258(18)88697-5)
4. Ignat, I., Volf, I., and Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4), 1821–1835. <https://doi.org/10.1016/j.foodchem.2010.12.026>
5. International Council for Harmonisation. (2022). *Validation of Analytical Procedures Q2(R2)*. ICH Harmonised Guideline. https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf
6. Khoddami, A., Wilkes, M. A., and Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328–2375. <https://doi.org/10.3390/molecules18022328>
7. Naczek, M., and Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054(1–2), 95–111. <https://doi.org/10.1016/j.chroma.2004.08.059>
8. Plazonić, A., Bucar, F., Maleš, Z., Mornar, A., Nigam, B. S., and Kujundžić, N. (2009). Identification and quantification of flavonoids and phenolic acids in *Crataegus* species using HPLC-DAD-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis*, 49(3), 599–603. <https://doi.org/10.1016/j.jpba.2008.11.026>
9. Plotka-Wasyłka, J., Rutkowska, M., Owczarek, K., Szymańska, K., Żwir-Ferenc, A., and Namieśnik, J. (2017). Extraction with green solvents in analytical chemistry. *Trends in Analytical Chemistry*, 91, 12–25. <https://doi.org/10.1016/j.trac.2017.03.006>
10. Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *Journal of Chromatography A*, 1000(1–2), 657–691. [https://doi.org/10.1016/S0021-9673\(03\)00058-X](https://doi.org/10.1016/S0021-9673(03)00058-X)
11. Rzeppa, S., and Briesen, H. (2011). Separation and identification of phenolic compounds in botanical extracts using reversed-phase HPLC coupled with mass spectrometry. *Phytochemical Analysis*, 22(4), 312–321. <https://doi.org/10.1002/pca.1281>
12. Singleton, V. L., and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>
13. Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*, 30(18), 3268–3295. <https://doi.org/10.1002/jssc.200700261>
14. Vrhovsek, U., Masuero, D., Gasperotti, M., Mattivi, F., and Franceschi, P. (2012). A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in biological samples. *Journal of Agricultural and Food Chemistry*, 60(36), 8831–8840. <https://doi.org/10.1021/jf2051569>
15. Vukics, V., and Guttman, A. (2010). Structural characterization of flavonoid glycosides by HPLC-MS. *Mass Spectrometry Reviews*, 29(1), 1–16.