Review

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Separation Strategies for Polyphenols from Plant Extracts: Advances, Challenges, and Applications

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Abstract

Polyphenols are a structurally diverse group of plant secondary metabolites widely recognized for their antioxidant, anti-inflammatory, antimicrobial, and chemoprotective properties, which have stimulated their extensive use in food, pharmaceutical, nutraceutical, and cosmetic products. However, their chemical heterogeneity, wide polarity range, and strong interactions with plant matrices pose major challenges for efficient extraction, separation, and reliable analytical characterization. This review provides a critical overview of contemporary strategies for the extraction, separation, and identification of polyphenols from plant-derived matrices. Conventional extraction methods, including maceration, Soxhlet extraction, and percolation, are discussed alongside modern green technologies such as ultrasound-assisted extraction, microwave-assisted extraction, pressurized liquid extraction, and supercritical fluid extraction. Particular emphasis is placed on environmentally friendly solvents, including ethanol, natural deep eutectic solvents, and ionic liquids, as sustainable alternatives that improve extraction efficiency while reducing environmental impact. The review further highlights chromatographic separation approaches—partition, adsorption, ion-exchange, size-exclusion, and affinity chromatography—and underlines the importance of hyphenated analytical platforms (LC–MS, LC–MS/MS, and LC–NMR) for comprehensive polyphenol profiling. Key analytical challenges, including matrix effects, compound instability, and limited availability of reference standards, are addressed, together with perspectives on industrial implementation, quality control, and standardization.

Keywords: polyphenols; plant extracts; green extraction technologies; chromatographic separation; LC–MS/MS; hyphenated analytical techniques; functional foods; nutraceuticals; dermocosmetics

1. Introduction

1.1. Polyphenols: Structural Diversity, Biological Significance, and Analytical Challenges

Polyphenols represent one of the most important and structurally diverse groups of bioactive compounds in plants, contributing significantly to their pigmentation, growth regulation, and defense biotic stress factors [1]. The secondary metabolites, includes phenolic acids, flavonoids, stilbenes, and tannins, have been extensively investigated for their beneficial effects on human health due to their antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [2]. As a result, polyphenols have gained considerable attention as natural therapeutic agents and key components in functional food, nutraceuticals, and dermocosmetic formulations [3,4]. However, the structural complexity and wide chemical diversity of polyphenols present significant analytical challenges for their isolation, separation, and quantification [5]. Their distribution in complex plant matrices, combined with susceptibility to oxidation and degradation, complicates the development of standardized analytical protocols [6]. Therefore, optimizing extraction and separation procedures has become a fundamental step in ensuring reproducibility and reliability of polyphenol analysis.Conventional techniques such as maceration and Soxhlet extraction have been widely used

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for decades but are often inefficient in terms of time, solvent consumption, and thermal degradation of sensitive compounds [7]. In contrast, modern "green" extraction technologies – including ultrasound –assisted extraction (UAE), microwave-assisted extraction (MAS), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) – offer faster, more efficient, and environmentally friendlyalternatives [8,9]. The introduction of natural deep eutectic solvents (NaDES) and ionic liquids as alternative extraction media represents another major step toward sustainable and non-toxic processing [10,11]. Parallel to extraction advances, the development of high-resolution analytical tools has transformed the study of polyphenols. High-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), and capillary electrophoresis (CE), combined with advanced detectors such as diode-array detection (DAD), mass spectrometry (MS), and nuclear magnetic resonance (NMR), enable detailed profiling of complex mixtures. Moreover, hyphenated and miniaturized systems (e.g., LC-MS/MS, LC-NMR, microchip CE) have facilitated rapid, sensitive, and precise quantification even in challenging plant matrices [6]. Thus, this review aims to summarize the state-of-the-art methodologies in polyphenol extraction and separation, highlight current analytical challenges, and discuss about their implications in the development of functional food, nutraceuticals, and dermocosmetic products.

1.2. Applications of Polyphenols in Food, Pharmaceutical, and Cosmetic Industries

The multifunctional nature of polyphenols has positions them at the forefront of research and industrial innovation in food, pharmaceutical, and cosmetic sciences. Their chemical diversity, combined with strong antioxidant, anti-inflammatory, and antimicrobial effects, provides a broad basis for practical applications that link human health benefits with product stability and quality [12,16]. In the food sector, polyphenols are primarily recognized as natural antioxidants that prevent lipid oxidation, inhibit enzymatic browning, and extend the shelf life of perishable products. Their inclusion in functional foods and beverages contributes to the prevention of oxidative stress-related diseases, including cardiovascular disorders, diabetes, and cancer [3]. Phenolic acids and flavonoids are frequently incorporated into cereal-based products, fruits juices, dairy alternatives, and dietary supplements to improve both nutritional and sensory properties [12]. In pharmaceutical formulations, polyphenols demonstrate a variety of pharmaceutical effects, including antioxidant, anti-inflammatory, cardioprotective, neuroprotective, and anticancer actions [2]. Flavonoids such as quercetin, catechins, and epigallocatechin gallate (EGCG) exhibit strong potential in modulating signaling pathways associated with inflammation and apoptosis [17]. Furthermore, resveratrol, curcumin, and catechins have been extensively studied for their ability to regulate gene expression, improve endothelial function, and suppress oxidative stress, which makes them promising agents for the prevention of metabolic and neurodegenerative diseases [18]. Drug delivery systems such as liposomes, polymeric nanoparticles, and solid lipid carriers are increasingly used to overcome the poor water solubility and low bioavailability of polyphenols, ensuring controlled release and improved therapeutic efficiency [19]. In cosmetic and dermocosmetic formulations, polyphenols are valued for their ability to protect skin from oxidative stress, photoaging, and inflammation induced by UV radiation and environmental pollutants. Their inclusion in topical formulations enhances skin elasticity, reduces pigmentation, and supports collagen synthesis [20]. Compounds such as resveratrol, catechins from green tea, anthocyanins, and phenolic acids are incorporated into creams, serums, and emulsions for their anti-aging, skin-brightening, and anti-tyrosinase effects [21]. Recent innovations have introduced delivery systems such as liposomes, nanoemulsions, and exosomes to improve penetration of polyphenols into deeper skin layers, enhancing both efficacy and stability [22]. The integration of polyphenols into "green cosmetics" aligns with the global trend toward sustainability and the replacement of synthetic antioxidants and preservatives with natural bioactives.

In summary, polyphenols serve as multifunctional biomolecules with proven roles in food preservation, therapeutic formulation, and cosmetic innovation. Their future applications will

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depend largely on the advancement of extraction, stabilization, and delivery technologies that preserve bioactivity while enabling scalable and eco-friendly production.

1.3. Challenges in the Extraction and Analysis of Polyphenols

Although polyphenols are among the most extensively studied phytoconstituents, their extraction and analysis remain a major challenge due to the great structural diversity and the complexity of plant matrices. These compounds encompass a wide spectrum of molecules differing in molecular weight, degree of polymerization, polarity, and stability, making it difficult to establish a universal procedure for their isolation and quantification [5,6]. One of the key issues in the extraction process is the optimization of conditions that ensure maximum yield while preventing degradation of thermolabile compounds. Parameters such as temperature, pH, solvent type and polarity, extraction time, and agitation rate have a profound effect on efficiency [23,24]. Traditional techniques, including maceration and Soxhlet extraction, are often time-consuming and require large volumes of organic solvents, which may lead to oxidation or hydrolysis of sensitive flavonoids and phenolic acids [10]. The introduction of "green" extraction technologies – ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) – has significantly improved extraction efficiency and the preservation of bioactive compounds. Nevertheless, no single technique is capable of recovering the full range of polyphenols from complex plant matrices [9]. The analytical characterization of polyphenols is also burdened with numerous difficulties. Standardization of identification and quantification methods is complicated by the lack of reference standards, the presence of isomeric or derivative forms, and the possible formation of artifacts during sample preparation. Widely used chromatographic methods such as high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC) provide good resolution but require careful optimization of mobile-phase composition, column type, and detection mode. Another major limitation arises from matrix effects–the interactions between polyphenols and other plant constituents which may cause signal suppression or enhancement, thus reducing the accuracy of quantification [6]. The application of hybrid analytical platforms, such as LC-MS/MS and LC-NMR, together with multivariate statistical approaches, has enabled a deeper understanding of polyphenol composition; however, these techniques are still complex, time-consuming, and costly [8]. Consequently, current research efforts are focused on developing integrated and environmentally sustainable analytical methodologies that combine high selectivity, minimal solvent consumption, and automated parameter control, providing improved reproducibility and reliability of analytical results.

1.4. Aim and Scope of This Review

The aim of this review is to provide a systematic overview of recent advancements and technological progress in the extraction, separation, and characterization of polyphenols, with particular emphasis on their relevance to the food, pharmaceutical, and cosmetic industries. Polyphenols represent highly complex and heterogeneous group of bioactive molecules, requiring multidisciplinary approaches both in their isolation and in their analytical profiling. Therefore, the focus of this work is on a critical evaluation of innovative techniques that offer enhanced selectivity, improved efficiency, and more reliable identification of individual phenolic compounds in complex biological matrices. This review further examines the progress achieved in classical and modern separation techniques, including HPLC, UHPLC, and high-resolution MS-based systems, as well as the role of integrated analytical platforms (e.g., LC-MS/MS, LC-HRMS, and multidimensional chromatography) in improving the structural elucidation of polyphenols. Particular attention is given to recent developments in advanced extraction approaches such as ultrasound-assisted and microwave-assisted extraction, supercritical fluid extraction, and green-chemistry-based techniques that contribute to enhanced efficiency, higher selectivity, and better preservation of the structural integrity of phenolic constituents. Challenges related to isomeric complexity, the limited availability

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of references standards, matrix effects, and potential degradation processes occurring during sample preparation. By addressing these aspects, the review provides an integrated perspective on how modern analytical technologies have improved our understanding of polyphenols and to what extent these methods have contributed to increased reliability, sensitivity, and precision in polyphenol research. Through this comprehensive overview, the review aims to offer researchers clear insights into the current state to the fields as well as guidance for selecting the most appropriate extraction and analytical strategies based on the structural, stability-related, and functional properties of the target polyphenolic compounds.

2. Overview of Major Plant Sources of Polyphenols

2.1. Natural Sources and Distribution of Polyphenols

Polyphenols are widely distributed throughout the plant kingdom, and their concentrations, structural diversity, and bioavailability vary greatly depending on botanical origin, physiological role, and environmental conditions. Understanding the principal natural sources of polyphenols is crucial for optimizing extracting strategies and developing high-value formulations intended for food, pharmaceutical, and cosmetic applications [19,25].

2.1.1. Fruits

Fruits are among the richest natural reservoirs of polyphenols, including anthocyanins, flavanols, flavan-3-ols, phenolic acid, and various tannins. Berries such as blueberries, blackberries, and raspberries typically exhibit high levels of anthocyanins and ellagitannins, whereas apples and pears contain significant amounts of chlorogenic acid and quercetin glycosides [1]. Grapes represent a particularly important source characterized by abundant catechins, procyanidins, and anthocyanins, whose distribution depends on cultivar, maturity, and processing conditions [26].

2.1.2. Wine

Wine-especially red wine-is a complex matrix rich in phenolic compounds derived from grape skins seeds, and stems. Its polyphenolic profile includes resveratrol, anthocyanins, tannins, flavanols, and phenolic acids, all of which contribute to color stability, oxidative balance, and sensory properties [27]. The phenolic content of wine is strongly influenced by vinification practices such as maceration duration, fermentation temperature, and yeast strain used.

2.1.3. Tea

Tea (Camellia sinensis) is one of the most important global dietary sources of phenolic compounds, particularly catechins including epigallocatechin gallate (EGCG), epicatechin (EC), and epicatechin gallate (ECG). Minimal oxidation during processing enables green tea to retain high concentrations of monomeric catechins, whereas black tea undergoes enzymatic fermentation, resulting in polymerized products such as theaflavins and thearubigins [28]. Due to their potent antioxidant, anti-inflammatory, and chemo protective effects, tea polyphenols remain a central focus of nutritional and pharmacological studies.

2.1.4. Medicinal Plants

Medicinal plants contain diverse and often highly concentrated polyphenolic profiles. Species such as *Hypericum perforatum*, *Rosmarinus officinalis*, *Matricaria chamomilla*, along with numerous members of the *Lamiaceae* and *Asteraceae* families, are rich in flavonoids, phenolic acids, and complex tannins [29]. These compounds are frequently associated with antioxidant, antimicrobial, hepatoprotective, and anti-inflammatory activities. However, the chemical heterogeneity of medicinal plants requires advanced extraction and analytical techniques for accurate characterization of bioactive fractions [30].

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2.1.5. Agro-industrial By-products

Agro-industrial by-products represent a sustainable, low-cost source of polyphenols and are increasingly important within circular economy concepts. Common examples include grape pomace, olive mill wastewater, citrus peels, apple pomace, and cereal bran. These materials can contain substantial concentrations of flavan-3-ols, hydroxycinnamic acids, hesperidin, naringin, oleuropein, and tannins [31].

2.2. Structural Classification of Polyphenols (Flavonoids, Phenolic Acids, Tannins, Stilbenes, Lignans)

Polyphenols constitute one of the most diverse classes of plant secondary metabolites, characterized by the presence of multiple hydroxyl groups attached rings. Their chemical heterogeneity reflects a wide spectrum of biosynthetic origins, structural features, physicochemical properties, and biological activities [32,33]. Based on their core molecular scaffolds, polyphenols are generally classified into five major groups: flavonoids, phenolic acids, tannins, stilbenes, and lignans. These classes differ in the configuration and substitution patterns of the aromatic rings, types of linkages, degree of polymerization, and presence of glycosidic or acyl derivatives.

2.2.1. Flavonoids

Flavonoids represent the most structurally diverse and biologically significant subgroup of polyphenols, comprising over 6,000 known compounds. They share a common C6-C3-C6 carbon skeleton formed by two aromatic rings (A and B) connected via a heterocyclic C-ring (flavan nucleus). According to the degree of oxidation and saturation of the C-ring, flavonoids are further divided into several subclasses: flavones, flavonols, flavanones, flavan-3-ols (catechins), anthocyanins, and isoflavones [25]. Flavnoids often occur as glycosides, which influence their solubility, stability, and bioavailability. For instance, quercetin predominantly appears as quercetin-3-O-rutinoside (rutin) in fruits, while anthocyanins exist almost exclusively in glycosylated forms to stabilize the flavylium cation. Biological activities include antioxidant, cardioprotective, anti-inflammatory, enzyme-modulating, antimicrobial, and neuroprotective effects [1]. Structurally, flavonoids possess high reactivity toward free radical due to the presence of catechol moieties and conjugated double bonds.

2.2.2. Phenolic Acids

Phenolic acids are a large group of simple, low-molecular-weight polyphenols divided into hydroxybenzoic acids (C6-C1) and hydroxycinnamic acids (C6-C3). Representative hydroxybenzoic acids include gallic, protocatechuic, syringic, and vanilic acids, whereas caffeic, p-coumaric, ferulic, and sinapic acid represent the hydroxycinnamic subclass [33]. These compounds frequently occur in free forms or as esterified derivatives such as chlorogenic acid (caffeoylquinic acid) and rosmarinic acid. Phenolic acids contribute to the sensory properties of plant foods (astringency, bitterness) and demonstrate strong antioxidant and antimicrobial activities [25]. Due to structural simplicity, phenolic acids are more amenable to quantification using chromatographic techniques (HPLC, UHPLC), although they are highly sensitive to oxidation and pH-induced degradation.

2.2.3. Tannins

Tannins are high-molecular-weight polyphenols capable of forming strong complexes with proteins and polysaccharides. They are broadly categorized into:

1. Hydrolyzable tannins – esters of gallic acid or ellagic acid with glucose (e.g., gallotannins, ellagitannins). These tannins readily hydrolyze under acidic or enzymatic conditions.
2. Condensed tannins (proanthocyanidins) – oligomers or polymers of flavan-3-ols (catechin, epicatechin, gallocatechin). Their polymerization degree strongly influences biological activity and extractability [33]

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Tannins exhibit potent antioxidant, anti-inflammatory, antimicrobial, and metal-chelating properties. They are particularly abundant in grape seeds, pomegranate, nuts, and tea. Due to their high structural variability, analytical characterization requires advanced MS-based platforms and often multidimensional chromatography.

2.2.4. Stilbenes

Stilbenes are a smaller but highly significant group of polyphenols characterized by a 1,2-diphenylethylene backbone. The most prominent compound is resveratrol (3,5,4′-trihydroxystilbene), widely present in grapes, berries, peanuts, and red wine [27]. Stilbenes often exist as cis/trans isomers and their biological activity is strongly influenced by configuration and glycosylation (e.g., piceid – resveratrol glucoside). They exert antioxidant, cardioprotective, antimicrobial, antiviral, and anti-aging effects. In plants, stilbenes function as phytoalexinsdefense compounds synthesized in response to stress.

2.2.5. Lignans

Lignans are phenolic dimers biosynthesized via the oxidative coupling of two coniferyl alcohol residues. Their core structure is typically a dibenzylbutane skeletion, although numerous derivatives exist [34]. Key lignans include secoisolariciresinol, matairesinol, pinoresinol, and laricisinol, abundant in flaxseed, sesame, whole grains, berries, and various medicinal plants. In humans, lignans are metabolized by gut microbiota to enterolignans (enterodiol and enterolactone), which show estrogenic and antiestrogenic properties. Lignans have been associated with anti-inflammatory, antioxidant, antitumor, and cardiometabolic benefits.

Table 1. Structural characteristics of major classes of polyphenols.

Class of polyphenols	Core structure	Key subgroups/ examples	Structural features	Main plant sources
Flavonoids	C6-C3-C6 (flavan nucleus)	Flavonols (quercetin), flavones (luteolin), flavan-3-ols (catechin),Anthocyanins (cyaniding), isoflavones (genistein)	Oxidation state of C-ring, glacosylation common	Fruits, tea wine, medicinal plants
Phenolic acids	Hydroxybenzoic (C6-C1) /Hydroxycinnamic (C6-C3)	Gallic acid, caffeic acid, ferulic acid, chlorogenic acid	Free or esterified forms	Fruits, cereals, coffee, vegetables
Tannins	Polymerized polyphenols	Condensed tannins (procyanidins), Hydrolyzable tannins (gallotannins)	High MW, protein-binding	Grapes, nuts, tea, pomegranate
Stilbenes	1,2-diphenylethylene	Resveratrol, piceid	Cis/trans isomerism	Grapes, berries, wine
Lignans	Dibenzylbutane-type dimers	Secoisolariciresinol, pinoresinol	Derived from coniferyl alcohol	Flaxseed, sesame, grains

Table 2. Major classes of polyphenolic compounds and their dominant biological activities.

Class	Dominant biological activities	References
Flavonoids	Antioxidant, anti-inflammatory, enzyme modulation, cardioprotective	[1]
Phenolic acids	Antioxidant, antimicrobial, antiinflamatory	[25]

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Tannins	Antioxidant, metal-chelating, antimicrobial, anti-inflammatory	[33]
Stilbenes	Antioxidant, cardioprotective, anti-aging, antimicrobial	[27]
Lignans	Anticancerogenic, estrogenic/antiestrogenic, cardiometabolic effects	[34]

2.3. Structure–Activity Relationship of Polyphenols

Understanding the relationship between chemical structure and biological activity represents a central concept in phytochemistry and nutritional biochemistry. Polyphenols comprise a structurally diverse family of compounds whose physiological effects are strongly dependent on the number and arrangement of hydroxyl groups, the degree of conjugation, polymerization, glycosylation patterns, and the ability to participate in complex interactions with metals, proteins, lipids, and other biomolecules [1,35]. One of the most important structural determinants of antioxidant potential is the presence of ortho-dihydroxy (catechol) groups on the B-ring. This arrangement facilitates the formation of a resonance-stabilized semiquinone radical, which significantly enhances reactivity toward free radicals. Flavonoids such as quercetin and catechin are therefore among the most potent natural antioxidants [35]. In addition, the conjugated C2=C3 double bond in combination with a 4-keto group in the C-ring of flavonols further enhances radical stabilization, explaining why flavonols generally exhibit stronger antioxidant properties than flavanones or flavones [25]. Structural modifications such as galloylation also enhance antioxidant and antimicrobial activity because galloyl groups contain additional phenolic OH groups capable of electron donation. Epigallocatechin gallate (EGCG), one of the most potent tea polyphenols, derives its exceptional antioxidant capacity from this structural feature [33]. Polyphenol-protein interactions provide another example of structure-driven biological activity. Tannins and proanthocyanidins with a high degree of polymerization exhibit a pronounced tendency to bind and precipitate proteins, accounting for their astringency and their antimicrobial, antiviral, and antifungal effects. Binding to microbial surface proteins can disrupt their structure and function, but increased polymerization simultaneously reduces bioavailability, meaning that highly polymerized tannins act primarily locally in the gastrointestinal tract, while systemic effects are mediated mainly by oligomeric derivatives [33]. Glycosylation – the most common modification of flavonoids in plants-has a marked influence on physicochemical and biological properties. Glycosides generally display greater solubility and stability but lower lipophilicity, which reduces membrane permeability and affects intestinal absorption. For example, quercetin aglycone is poorly soluble but more biologically active at the cellular level, whereas its glycoside rutin is more soluble and stable but less readily absorbed [32]. Among anthocyanins, acylation significantly increases stability toward pH changes, light, and temperature, a property of particular importance in food technology and colorant applications. Subclasses of polyphenols also exhibit unique structure-dependent traits. It stilbenes, cis/trans isomerism is major determinant of biological activity. Trans-resveratrol is more stable and demonstrates stronger antioxidant and anti-inflammatory properties than the cis-isomer. Glycosylated derivatives, such as piceid, exhibit greater stability but lower immediate biological activity [27]. Finally, a crucial dimension of structure-activity relationships involves microbial biotransformation. Many polyphenols-especially lignans-undergo extensive modification by the gut microbiota before exerting physiological effects. Lignans such as secoisolariciresinolare converted into enterodiol and enterolactone, which possess pronounced estrogenic, anti-inflammatory, and antioxidant properties [34]. This illustrates that the bioactivity of polyphenols is not governed coley by their native structure but also by their metabolites and overall bioavailability.

Table 3. Structural characteristics and their influence on polyphenol bioactivity.

Structural feature	Influence on bioactivity	Examples
Ortho-dihydroxy groups	High antioxidant activity; metal chelation	Quercetin, catechn

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Conjugated C2=C3 bond + 4-keto group	Radical stabilization; increased antioxidant potential	Flavonols
Gelloylation	Strong radical scavenging: enhanced bioactivity	EGCG
High degree of polymerization	Protein binding; antimicrobial activity; reduced absorption	Tannis
Glycosylation	Increased stability and solubility; reduced lipophilicity	Anthocyanins, rutin
Acylation	Improved pH and thermal stability	Acylated anthocyanins
Cis/trans isomerism	Differences instability and efficacy	Trans-resveratrol
Microbial biotransformation	Formation of active metabolites	Lignans→enterolactone

3. Extraction Methods for Polyphenols

3.1. Conventional Extraction Methods for Polyphenols: Maceration, Soxhlet Extraction, and Percolation

Conventional extraction techniques represent the oldest and most widely applies methods for the isolation and separation of polyphenols from plant materials. Despite the advancement of modern techniques such as accelerated solvent extraction, ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction, traditional approaches remain extensively used due to their simplicity, low equipment requirements, reproducibility, and the availability of vast comparative literature. Among these, maceration, Soxhlet extraction, and percolation are the most commonly utilized procedures, each differing in mass transfer mechanisms, extraction kinetics, and efficiency. The selection of a suitable method depends largely on the nature of the plant matrix, solvent properties, and the stability of the target polyphenols.

Maceration is a static process based on the diffusion of soluble compounds from plant tissues into a solvent under ambient or mildly elevated temperatures. It is especially suitable for thermolabile polyphenols such as anthocyanins, ellaginates, catechins, and various times, maceration provides excellent preservation of native poyphenolic structures, making it advantageous for matrices rich in heat-sensitive metabolites. Numerous studies have demonstrated the successful recovery of catechins from green tea, punicalagis from pomegranate peel, and anthocyanins from berry fruits, with minimal degradation of phenolic compounds [26,36].

In contrast, Soxhlet extraction is a dynamic technique that enables continuous process in which fresh solvent percolates through a packed bed of plant material with freshly condensed hot solvent at the boiling point. Owing to repeated solvent recirculation and constant renewal of the extraction medium, Soxhlet extraction achieves a high degree of matrix exhaustion and typically yields higher quantities of polyphenols compared with static maceration. This method is particularly effective for extracting more thermally stable polyphenols, such as stilbenes, phenolic acids, and certain flavonoids. However, elevated temperatures may cause degradation of heat-sensitive metabolites, posing a limitation for analytes like anthocyanins and some tannins. Despite this, Soxhlet remains a reference technique for the extraction of rosmarinic acid from rosemary, hesperidin from citrus peels, and resveratrol from grapes, and is frequently cited as a benchmark in extraction studies [37,38]. Percolation, the third major conventional method, is a continuous process in which fresh solvent percolates through a packed bed of plant material, providing improved extraction kinetics and a steep diffusion gradient compared with static maceration. This technique is particularly suitable for scale-up and industrial applications, especially in the production of tinctures, botanical extracts, and phytopharmaceuticals. Percolation has demonstrated excellent performance in extracting phenolic acids from sage, fravonoid aglycones from ginkgo leaves, and hypericin and rutin-type flavonoids from St. John's wort. Because of its dynamic nature, percolation often results in higher yields than maceration while maintaining the structural integrity of sensitive polyphenols [30,39]. Overall, the choice between these methods depends on the chemical characteristics of the target phenolic

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compounds, including their stability, polarity, and susceptibility to degradation. Conventional techniques remain essential in phytochemical research, serving both as primary extraction approaches and as reference methods for validating more advanced technologies.

Table 4. Extraction methods, plant materials, and polyphenolic compounds identified using chromatographic techniques.

Extraction Method	Plant Material	Solvent & Condition	Extracted Polyphenols	Analytical Technique	Reference
Maceration	*Cmellia sinensis* (green tea)	70% ethanol, 25°C, 48h	Catechin, Epicatechin, EGCG	HPLC-DAD	[23,36]
Maceration	Punica granatum (pomegranate Peel)	80% methanol	Punicalagin, ellagic acid	HPLC	[40]
Maceration	Vaccinium myrtillus (bilberry)	Methanol + 1% HCL	Anthocyanins (malvidin, delphinidin)	LC-MS/MS	[23]
Soxhlet	Rosmarinus officinalis	Ethanol, 78°C, 6h	Rosmarinic, ferulic acid	HPLC	[37]
Soxhlet	Citrus limon (lemon peel)	Methanol	Hesperidin	UHPLC	[41]
Soxhlet	Vitis vinifera	Ethyl acetate	Resveratrol, piceatannol	HPLC	[18,42,43]
Percolation	Salvia officinalis	70% ethanol	Caffeic and rosmarinic acid	HPLC	[44]
Percolation	Ginkgo biloba	Acetone:water (60:40)	Quercetin, Kaempferol, isorhamnetin	HPLC-DAD/MS	[39]
Percolation	Hypericum perforatum	80% ethanol	Hypericin, rutin, quercetin	LC-MS	[30]

3.2. Modern and "Green" Extraction Techniques for Polyphenols

Modern extraction technique based on the principles of green chemistry have been developed as a response to the limitations of conventional methods, which typically request large volumes of solvents, prolonged processing times, and often negatively affect the stability and integrity of polyphenolic compounds. Among the most important techniques currently used in phytochemical, pharmaceutical, and food-related research are ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE). These techniques enable more efficient isolation of polyphenols with reduced solvent consumption, lower energy input, and improved preservation of thermolabile compounds, making them highly suitable for modern analytical applications and the development of natural products [36,45].

3.2.1. Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is based on the phenomenon of acoustic cavitation, where ultrasonic waves induce the formation, growth, and implosion of microbubbles in the solvent. The implosion generates extreme localized conditions, including high pressure and temperatures, which disrupt cell walls and significantly accelerate the diffusion of intracellular metabolites. This mechanism makes UAE one of the most efficient and widely used green techniques for polyphenol extraction. Literature reports indicate that UAE can reduce solvent consumption by 30-50% comparation to conventional maceration, while extraction time is shortened from several hours to only 5-30 minutes [24]. UAE is particularly suitable for thermolabile compounds because extraction

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can be performed at low temperatures, typically below 50 °C. UAE has been successfully applied for the isolation of resveratrol from grape pomace, resulting in up to threefold higher yield compared with Soxhlet extraction [38]. It has also provided highly effective for extracting flavonoids such as quercetin and catechins from onion and green tea, as well as anthocyanins from blueberries, achieving significantly improved yields in shorter processing times. These advantages make UAE a method of choice for both laboratory-scale and industrial-scale photochemical applications [46].

3.2.2. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction employs microwave energy to rapidly heat the solvent and plant matrix. Selective heating of polar molecules leads to increased pressure inside plant cells, causing cell rupture and the efficient release of intracellular constituents. This technique provides a very short extraction time–often up to 90% shorter than Soxhlet extraction-while maintaining the stability of thermolabile phenolic compounds due to brief exposure to elevated temperatures [47]. MAE has proven particularly effective for extracting stilbenes and flavonols from grape and grape-processing by-products. Studies have shown that MAE performed at 60 °C using ethanol as a solvent yield up to 2.5 times more resveratrol compared with conventional methods. High extraction efficiencies have also been reported for caffeic and ferulic acids from cereals, as well as for flavonols such as quercetin and myricetin from grape skins, demonstrating the broad applicability of MAE in the separation of polyphenols for nutritional, pharmaceutical, and cosmetic purposes [48,49].

3.2.3. Pressurized Liquid Extraction (PLE/ASE)

Pressurized liquid extraction (PLE) also known as accelerated solvent extraction (ASE), employs solvents at elevated temperatures (50-200 °C) and pressures (10-15 MPa), maintaining the solvent in a liquid state even above its boiling point. Under these conditions, solvent viscosity and surface tension decrease, while solvent penetration and solubilization power increase, resulting in significantly more efficient extraction of polyphenols. PLE is considered one of the most reproducible and highly automated green extraction methods, characterized by low solvent consumption and short extraction times, typically ranging from 5 to 20 minutes. Studies confirm that PLE markedly increases the yield of polyphenols from various matrices. For example, extraction of flavonoids from grape pomace at 150 °C resulted in a ~40% increase in yield compared with conventional methods [42]. Subcritical water within the PLE domain is particularly effective for isolating phenolic acids such as vanilic and p-coumaric acid from red pepper and berry fruits [50].

3.2.4. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction is one of the most environmentally friendly techniques for isolating bioactive compounds. The most commonly used fluid is supercritical carbon dioxide ($scCO_2$), which in its supercritical state (T> 31.1 °C, P > 7.38 MPa) possesses gas-like diffusivity combined with liquid-like solvating power. These properties enable highly selective extraction of lipophilic molecules, but also polyphenols when CO_2 is modified with co-solvent, typically ethanol at concentrations of 5-15% [51]. SFE is distinguished by the exceptionally high purity of the extracts produced, as CO2 easily transitions into the gaseous phase after depressurization, leaving the extract completely free of solvent residues. Low extraction temperatures (35-50 °C) further protect thermolabile polyphenols, making SFE particularly suitable for isolating antioxidants and high-value phytochemicals. Studies show that SFE with ethanol as a co-solvent significantly increases the yield of stilbenes and flavonols from grape skins, while the technique is also widely used for the extraction of phenolic diterpenes such as carnosol and carnosic acid from rosemary.

Table 5. Comparative overview of modern extraction techniques for polyphenols.

Plant material	Method	Target polyphenols	Extraction conditions	Outcome

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Grape pomace	UAE	Resveratrol	40 kHz, 25-40 °C	3× higher yield
Grape skins	MAE	Quercetin, myricetin	60 °C, Ethanol	2-3× higher yield
Grape pomace	PLE	Flavonoids, phenolic acids	150°C, 10 MPa	+40% yield
Grapes, rosemary	SFE	Stilbenes, diterpenes	250-300 bar, 10%	High-purity extracts

3.2.5. Use of Environmentally Friendly Solvents

The increasing demand for sustainable extraction processes in the food, pharmaceutical, and cosmetic industries has stimulated the development and application of environmentally friendly solvents. Traditional organic solvents such as methanol, acetone, or chloroform are often associated with toxicity, volatility, and environmental burden. In contrast, green solvents–including ethanol, natural deep eutectic solvents (NaDES), and ionic liquids (ILs)–provide safer and more sustainable alternatives for extracting polyphenols and other bioactive compounds. These solvents exhibit higher biocompatibility, reduced toxicity, tunable physicochemical properties, and compatibility with green extraction techniques such as UAE, MAE, PLE, and SFE [32,46].

3.2.6. Ethanol as a Green Solvent

Ethanol is one of the most widely accepted green solvent due to its low toxicity, biodegradability, ease of recovery, and regulatory approved for use in food and pharmaceutical products. As a polar protic solvent, ethanol effectively solubilizes many classes of polyphenols, including phenolic acids, flavonoids, flavonols, stilbenes, and anthocyanins. Its extraction efficiency can be further optimized by using ethanol-water mixtures, in which the polarity can be fine-tuned to improved selectivity and yield [45].Numerous studies have demonstrated that ethanol-based extractions can outperform extractions preformed with methanol or acetone in terms of both efficiency and safety. For example, ethanol-water mixtures (50-70%) have been shown to enhance the extraction of resveratrol, catechins, and quercetin derivates from grape pomace and grape skins [49]. Ethanol has also been successfully employed in UAE and MAE, where the synergy between solvent polarity and extraction energy inputs further increases mass transfer and improves extraction yields. In industrial applications, ethanol is preferred not only because it is safe for human consumption, but also because it can be recovered through distillation, minimizing waste and making the entire extraction process more economical and environmentally compliant.

3.2.7. Natural Deep Eutectic Solvents

Natural deep eutectic solvents (NaDES) represent a new generation of green solvents formed by mixing hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) derived from natural compounds such as sugars, organic acids, amino acids, and polyols. These components interact through hydrogen bonding to form a eutectic mixture with a melting point significantly lower than that of the individual components [10]. NaDES are biodegradable non-toxic, non-volative, and structurally tunable, making them highly suitable for extracting sensitive phytochemicals. One of the key advantages of NaDES is their ability to create highly functionalized solvent environments capable of stabilizing polyphenols during extraction and storage. NaDES composed of choline chloride and organic acids (e.g., lactic or citric acid) have been particularly effective for extracting anthocyanins, chlorogenic acid, and catechins from berries, tea leaves, and fruit pomace [52]. Studies indicate that NaDES often outperform conventional ethanol-water mixture due to their stronger hydrogen-bonding networks, which enhance solvation of phenolic hydroxyl groups. Moreover, NaDES display excellent compatibility with UAE and MAE, where their enhanced viscosity can be mitigated through gentle heating or water dilution. Their applicability extends to food-grade formulations, cosmetics, and pharmaceutical preparations, particularly in cases where solvent residues remain in the final product, since NaDES components are typically recognized as safe (GRAS).

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3.2.8. Ionic Liquids

Ionic liquids (ILs), defined as salts with melting points below 100 °C, have gained significant attention as designer solvents for polyphenol extraction. ILs consist of bulky organic cations (e.g., imidazolium, pyridinium, ammonium) paired with inorganic or organic anions (e.g., chloride, acetate, tetrafluoroborate). Their tunable polarity, negligible vapor pressure, high thermal stability, and strong solvating ability make them powerful tools for extracting a broad range of phytochemicals [53]. Hydrophilic ionic liquids, particularly imidazolium-based ILs, have shown exceptional efficiency in solubilizing phenolic acids and flavonoids, often surpassing conventional solvents. For instance, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) has been reported to significantly enhance the extraction of rutin, quercetin, and caffeic acid from herbal matrices [38]. The strong solvation power of ILs arises from their ability to disrupt cell walls and form specific interactions with phenolic hydroxyl groups. However, despite their efficiency, the environmental impact and biodegradability of ILs remain topics of debate.

Table 6. Characteristics of environmentally friendly solvents used for polyphenols extraction.

Solvent type	Source/composition	Environmental Profile	Advantages	Limitations	Typical polyphenols extracted
Ethanol	Renewable (fermentation)	Biodegradable, low toxicity	Food-grade, Recoverable, tunable polarity	Lower solubility for highly hydrophobic compounds	Flavonoids, Phenolic acids, Stilbenes
NaDES	Sugars, amino acids	Biodegradable, nonvolatile	Highly tunable, stabilizing effect. GRAS components	High viscosity. Sometimes difficult removal	Anthocyanins, catechins, chlorogenic acid
Ionic liquids	Organic cations + inorganic/organic anions	Low vapor pressure; biodegrability varies	Highly tunable, excellent solvating power	Cost, potential toxicity, complex recycling	Rutin, quercetin, caffeic acid

The development of more environmentally safe ionic liquids-such as choline-based ILs and amino-acid-derived ILs-has emerged as an important direction. These “greener” ILs aim to maintain high extraction efficiency while reducing ecotoxicity and improving biodegradability [54]. ILs have been successfully integrated with MAE, enabling shorter extraction times and higher selectivity. Additionally, they have been applied in solid-liquid extraction of polyphenols from agro-industrial byproducts, including grape seeds, pomegranate peels, and olive leaves.

4. Separation and Purification Chromatographic Techniques for Polyphenols Identification

4.1. Fundamental Principles of Chromatography

Chromatography represents a group of analytical methods used for separating components of mixtures and, when coupled with appropriate detection techniques, also enables their qualitative and quantitative analysis. This has been significantly enhanced by the introduction of state-of-the-art detectors supported by advanced software packages, which have emerged from the rapid development of electronics over the past several decades. The term chromatography originates from the Greek words chroma (color) and graphein (to write), reflecting the earliest applications of the method to the purification of colored solutions.

Table 7. Recent Studies on Identification of Polyphenols by Chromatographic Methods.

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Identified Polyphenols	Chromatographic Method	Plant Source	Citation
Quercetin, Kaempferol, Rutin, Catechin	UHPLC-DAD-MS/MS	Vitis vinifera (Grape skin)	[58]
Chlorogenic acid, Caffeic acid, Ferulic acid	HPLC-ESI-QTOF-MS	Coffea arabica	[59]
Epicatechin, Procyanidin B1, B2	UHPLC-MS/MS	Camellia sinensis (Tea)	[60]
Naringenin, Hesperidin, Eriodictyol	LC-MS/MS	Citrus sinensis (Orange peel)	[61]
Malvidin-3-O-glucoside, Petunidin-3-O-glucoside	UHPLC-ESI-MS	Red grapes	[62]
Isorhamnetin, Quercetin-3-O-glucoside	HPLC-QTOF-MS	Hippophae rhamnoides	[63]
Catechin, Epicatechin gallate, Gallocatechin	UHPLC-MS/MS	Theobroma cacao	[64]
Kaempferol-3-O-rutinoside, Astragalin	UHPLC-MS	Moringa oleifera	[65]
Rutin, Hyperoside, Isoquercitrin	HPLC-DAD-MS	Hypericum perforatum	[66]
Chlorogenic acid derivatives	LC-QTOF-MS	Lonicera japonica	[67]
Genistein, Daidzein, Formononetin	HPLC-MS/MS	Trifolium pratense	[68]
Anthocyanins (Delphinidin, Cyanidin)	UHPLC-MS	Vaccinium myrtillus	[69]
Epigallocatechin-3-gallate	UHPLC-Orbitrap-MS	Camellia sinensis	[70]
Rosmarinic acid, Caffeic acid	HPLC-ESI-QTOF-MS	Salvia officinalis	[71]
Quercetin derivatives, Myricetin	UHPLC-MS	Allium cepa	[72]
Resveratrol, Piceid	UHPLC-MS/MS	Polygonum cuspidatum	[73]
Polymeric procyanidins	LC-QTOF-MS	Malus domestica	[74]
Phenolic acids, Flavonols	UHPLC-MS/MS	Rubus fruticosus	[75]

Samples intended for chromatographic analysis may consist of mixtures whose components can be chemically similar or highly diverse [55]. The sample that needs to be separated is firstly dissolved in a suitable solvent, after which this solution is transported by the flow of the mobile phase (a solvent or a solvent mixture) through the stationary phase, which is the basis of liquid chromatography. Upon contact with the stationary phase, each component interacts with it individually, usually through mechanisms such as distribution into the stationary phase, adsorption onto its surface, or other specific types of interactions. As a result of these interactions, the components become spatially separated: those with weak interaction with the stationary phase move nearly at the velocity of the mobile phase, whereas those with stronger interactions lag behind. For example, the samples of black currant, raspberry, honeysuckle, blueberry, and strawberry leaf extracts were analyzed by LC-ESI/MS and HPLC-DAD systems showed very rich sources of flavonols and hydroxycinnamic acid conjugates as well as many other compounds detected in the leaves with antiviral, antimicrobial, antidiabetic, and antioxidant activities [56]. When the mobile phase is in the gaseous state, and the sample must be volatilized before entering the stationary phase, the technique is referred to as gas chromatography [57].

When performed properly, the chromatographic process causes only negligible loss of sample, and when necessary, quantitative collection of separated components is possible; this is known as preparative chromatography. Modern chromatographic techniques offer exceptionally high sensitivity, enabling the separation and analysis of extremely small quantities of analytes (down to approximately 10-9 g or less). Thus, chromatography used primarily for partial or complete separation of mixture components is termed preparative chromatography [76], whereas chromatography used for qualitative and quantitative determination is known as analytical

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chromatography. Depending on the nature of interactions between analytes and the stationary phase, chromatographic techniques are classified as follows:

4.1. Partition Chromatography

When the stationary phase is a liquid immobilized on a solid support, analyte molecules can distribute between the stationary and mobile phases. The degree of distribution for each component is expressed by the partition coefficient K, defined as the ratio of its concentrations in the stationary (c_s) and mobile phases (c_m), at a given temperature and pressure:

$$K = (c_s/c_m)_{T,P}$$

A component that dissolves more readily in the stationary phase has a higher partition coefficient, moves more slowly through the column, and exhibits a longer retention time. Partition chromatography is conceptually similar to extraction and can be considered as a series of successive extractions between two immiscible phases. Partition chromatography, predominantly implemented as reversed-phase high-performance liquid chromatography (RP-HPLC), remains a central analytical tool for the identification of polyphenols in complex natural matrices due to its separation mechanism based on differential partitioning between a non-polar stationary phase and a polar mobile phase. This approach has been effectively applied for the comprehensive profiling of grape and wine polyphenols, where flavan-3-ols, phenolic acids, stilbenes, and anthocyanins were successfully identified using RP-HPLC-DAD-MS/MS in red wines and grape skins [58]. In tea analysis, RP-HPLC enabled the reliable identification of epigallocatechin gallate, epicatechin, rutin, and caffeic acid, confirming its relevance in functional beverage quality control [60]. Partition-based separation has also been widely employed for polyphenol characterization in propolis, allowing the identification of pinocembrin, galangin, chrysin, and caffeic acid derivatives by RP-HPLC-MS [77,78]. Furthermore, RP-HPLC partition mechanisms are routinely applied in medicinal plant analysis, such as Hypericum perforatum, where chlorogenic acid, hyperoside, rutin, and quercetin were identified in standardized extracts [66], as well as in berry fruits, where ellagitannins, flavonols, and anthocyanins were profiled with high selectivity [79]. These recent applications clearly demonstrate that partition chromatography remains a robust, versatile, and indispensable technique for reliable polyphenol identification across food, pharmaceutical, and nutraceutical matrices.

4.2. Adsorption Chromatography

In adsorption chromatography, separation is achieved based on differing adsorption affinities of mixture components. If the stationary phase is solid, analyte molecules can adsorb to and desorb from its surface. Components with a stronger affinity toward the stationary phase migrate more slowly and have longer retention times. Adsorption chromatography represents an important and frequently applied technique in the isolation and identification of polyphenols due to the strong interactions between phenolic hydroxyl groups and polar stationary phases such as macroporous resins, silica gel, polyamide, and cross-linked agarose gels. Wang and collaborators (2021) demonstrated the efficient adsorption and desorption of polyphenols from *Eucommia ulmoides* leaves using macroporous resins, achieving high enrichment of chlorogenic acid derivatives prior to analytical identification [80]. Similarly, Hou and collaborators (2021) applied macroporous adsorption resin for the purification of polyphenols from *Vernonia patula*, obtaining flavonoid- and phenolic-acid-rich fractions that were subsequently identified using HPLC-based techniques [81]. Rivera-Tovar and collaborators (2022) investigated the adsorption behavior of low-molecular-weight food-relevant polyphenols such as gallic acid, catechin, and resveratrol on cross-linked agarose gels, demonstrating that adsorption isotherms directly influence chromatographic separation selectivity [82]. Furthermore, Nasiru and collaborators (2024) successfully combined ultrasound-assisted extraction with silica-gel adsorption chromatography and RP-C18 purification to isolate and identify antioxidant polyphenols from *Cynanchum auriculatum* by LC-MS/MS and NMR [83]. These recent

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studies clearly confirm that adsorption chromatography remains a robust and indispensable tool for the preparative separation and reliable identification of polyphenols in complex natural matrices.

4.3. Ion-Exchange Chromatography

Some stationary phases contain covalently bound ionic functional groups. When analytes are present in ionic form, they can reversibly bind to these charged groups. Since analytes differ in their affinities for the ion-exchange sites, their separation is achieved by this principle.Its separation mechanism is based on reversible electrostatic interactions between ionized polyphenols and oppositely charged functional groups on the stationary phase, such as sulfonated cation-exchange or quaternary ammonium anion-exchange resins. Man and collaborators (2022) successfully applied strong anion-exchange chromatography for the purification and identification of phenolic acids from pomegranate peel, enabling selective fractionation of gallic, ellagic, and caffeic acids prior to RP-HPLC-MS confirmation [84]. Similarly, Marques (2025) demonstrated the effective use of cation-exchange chromatography for the isolation of anthocyanins from purple sweet potato, achieving high purity fractions that were subsequently identified by HPLC-DAD-MS [85]. Ion-exchange mechanisms have also been applied in complex plant matrices, as shown by dos Santos, (2021), who combined anion-exchange chromatography with LC-MS/MS for the selective enrichment and identification of hydroxycinnamic acids and flavonoid glycosides from green coffee beans [86]. These examples clearly illustrate that ion-exchange chromatography remains a powerful and highly selective tool for the separation, pre-concentration, and reliable identification of ionizable polyphenols.

4.4. Gel-Exclusion (Size-Exclusion) Chromatography

When analytes differ in molecular size, they pass through a porous stationary phase at different rates without adsorption or chemical binding. The stationary phase behaves like a molecular sieve, allowing smaller molecules to enter pores and migrate more slowly. This technique is widely applied for the separation of natural and synthetic polymers. The separation principle is based primarily on differences in hydrodynamic volume, allowing large polyphenols to elute earlier than smaller phenolic acids or flavonoid monomers, while the eluates are subsequently profiled by HPLC–DAD or LC–MS to assign individual structures. For example, Tian and collaborators (2018) used Sephadex LH-20 SEC to fractionate aqueous–ethanolic extracts of Finnish berry leaves and berries, obtaining size-resolved fractions enriched in (+)-catechin, (−)-epicatechin, quercetin glycosides and anthocyanins that were then identified by UPLC–DAD–ESI–MS and NMR [87]. Tenório and collaborators applied Sephadex LH-20 SEC to an ethyl-acetate fraction of Eugenia uniflora leaves, generating subfractions in which phenolic acids, flavonoid glycosides, aglycones and tannins were separated according to apparent molecular size and then structurally characterized by LC–ESI–HRMS/MS, linking specific polyphenols (e.g., myricitrin, quercetin and kaempferol derivatives) with anti-Candida activity [88]. In sorghum, Santana et al. developed a Sephadex LH-20 SEC protocol that cleanly separated high-molecular-weight condensed tannins from low-molecular-weight phenolic acids and flavonoids; subsequent LC analyses enabled characterization of the proanthocyanidin-rich fractions and demonstrated that these larger polyphenols exhibited stronger antiglycation and anticancer activities than the smaller species. Gel-exclusion chromatography with Sephadex LH-20 has also been used preparatively to isolate antioxidant-rich polyphenol fractions from Alternanthera sessilis Red, followed by HPLC–QTOF–MS/MS identification of chlorogenic acid derivatives and flavonoids in the size-resolved fractions [89]. Recent reviews on phenolic separation techniques highlight SEC on dextran-based gels such as Sephadex LH-20 as a robust option for deconvoluting complex plant extracts into size-homogeneous fractions prior to detailed LC–MS profiling, thereby improving both the qualitative and quantitative identification of polyphenols in food, nutraceutical and phytopharmaceutical matrices [90].

4.5. Specific Interactions – Affinity Chromatography

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Affinity chromatography is based on highly specific interactions between analytes and the stationary phase—most commonly involving biological molecules. Typical examples include antigen–antibody or enzyme–substrate interactions. Because of their high specificity, affinity systems allow extremely selective and efficient separations. Affinity chromatography represents a highly selective chromatographic approach for the identification and enrichment of polyphenols based on specific molecular recognition mechanisms such as enzyme–inhibitor, ligand–receptor, metal–phenolic coordination, and hydrogen-bond interactions. In this technique, biologically or chemically active ligands are immobilized onto a solid support, allowing only polyphenols with complementary binding affinity to be retained and selectively eluted. Affinity-based separation strategies have been increasingly employed for the targeted screening and identification of bioactive polyphenols from complex plant matrices. For instance, Ge and collaborators (2017) successfully applied tyrosinase-based affinity ultrafiltration coupled with LC–MS to selectively enrich and identify tyrosinase-binding ligands from *Pueraria lobata* extracts, demonstrating the effectiveness of enzyme–ligand interactions for targeted polyphenol discovery [91]. Similarly, Yin and collaborators (2019) utilized an ultrafiltration LC–MS approach combined with molecular docking to screen potential tyrosinase inhibitors from Semen Oroxyli, enabling the identification of flavonoids with strong enzyme-binding affinity [92]. Affinity chromatography and related bio-affinity techniques have also been extensively applied for the discovery of α-glucosidase-inhibitory polyphenols. Deng and collaborators (2014) developed an immobilized α-glucosidase affinity capture method coupled with UHPLC–QTOF–MS, which allowed the selective isolation of inhibitory catechins from green tea extracts [93]. More recently, Li and collaborators (2022) employed bio-affinity ultrafiltration combined with HPLC–ESI-qTOF-MS/MS to enrich and characterize α-glucosidase inhibitors from *Cerasus humilis* leaf tea, confirming the suitability of affinity-based methods for complex botanical samples [94]. In addition, Shi and collaborators (2022) applied a similar strategy to pomegranate peel extracts, enabling the targeted identification of phenolic compounds with strong α-glucosidase inhibitory potential [95]. Collectively, these studies demonstrate that affinity-based chromatographic and ultrafiltration techniques, relying on highly specific molecular recognition mechanisms, represent powerful and selective tools for the targeted identification of bioactive polyphenols in food, plant, and nutraceutical matrices.

Chromatography can also be classified according to the arrangement of the stationary phase:

1. Planar chromatography (thin-layer chromatography, paper chromatography) – separation occurs on a flat surface.
2. Column chromatography – separation occurs within a column packed or coated with the stationary phase.

Column chromatography is one of the most widely used preparative strategies for the purification and isolation of polyphenols from complex plant and food matrices. In open- or medium-pressure column formats, crude extracts are typically loaded onto packed beds of silica gel, dextran-based gels such as Sephadex LH-20, or synthetic macroporous resins, and phenolic compounds are eluted using polar organic–aqueous solvent systems. For example, Tian and collaborators (2018) fractionated aqueous ethanolic extracts of Finnish berry leaves and berries on Sephadex LH-20 columns, obtaining phenolic-rich fractions whose individual flavonoids and other phenolics were subsequently identified by UPLC–DAD–ESI–MS and NMR, and linked to antioxidant and antibacterial activities [87]. Macroporous adsorption resins have been extensively applied for column-based enrichment of polyphenols; Cao and collaborators (2024) developed an optimized chromatographic process using NKA-II resin to purify phenolics from Plantago depressa, and quantified six major phenolic compounds in the purified fractions by UPLC–TQ–MS/MS [96]. A similar strategy was used by Guo and collaborators who screened several macroporous resins and established a fast and efficient column protocol for the purification of polyphenols from kiwi fruit peel extracts, followed by HPLC analysis of the enriched fractions [97]. Beyond specific case studies, Mottaghipisheh and Iriti (2020) provided a comprehensive review on the use of Sephadex LH-20 column chromatography for the isolation and purification of flavonoids from a wide range of plant

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species, highlighting its effectiveness for obtaining quercetin and related derivatives in high purity [98]. More broadly, a recent review by Susanti et al. summarized column chromatography (including Sephadex LH-20, polyamide, silica gel and macroporous resin columns) as a core step in the multistage isolation workflows used to recover antioxidant phenolic compounds from plant extracts, often prior to final polishing by HPLC or other high-resolution techniques [90]. Collectively, these examples show that conventional column chromatography remains a versatile, scalable and cost-effective tool for producing polyphenol-enriched fractions and isolating individual phenolic constituents for structural and bioactivity studies. The modes of analysis in column chromatography include:

- Frontal analysis (continuous introduction of the analyzed solution into the column, where the least strongly "bound" substances move rapidly through the column and appear first in the effluent, i.e., in the eluate collected during elution, followed by the remaining components); For example, Snyder (2011) demonstrated the use of frontal analysis to characterize adsorption behavior of phenolic acids and flavonoids on silica and polymeric adsorbents, providing insight into competitive adsorption phenomena relevant for polyphenol separations [99]. Similarly, frontal chromatographic approaches have been applied to evaluate the interaction of catechins and simple phenolic acids with reversed-phase and normal-phase sorbents, aiding in the selection of optimal stationary phases for plant extract fractionation.
- Displacement analysis (only a small volume of the sample solution is introduced at the top of the column, after which the chromatogram is developed by passing either a solvent or a dissolved substance with a higher affinity for the stationary phase than any component of the analyzed mixture; this displacer forces all adsorbed substances to move, displacing one another); Although less frequently used for routine analysis, displacement chromatography has been successfully applied in preparative-scale isolation of plant polyphenols, particularly when high loading capacity and enrichment are required. For instance, displacement strategies using strongly adsorbing solvents or modifiers have been reported for the separation of flavonoid glycosides and aglycones from *Ginkgo biloba* and *Camellia sinensis* extracts, enabling enrichment of quercetin and kaempferol derivatives prior to further purification by elution chromatography or HPLC [100].
- Elution method (the most commonly used method of analysis, in which dissolved substances bind to the stationary phase from a small volume of solution at the top of the column and are then washed out with a pure solvent or a mixture of solvents—the eluent—under continuous flow). This mode is extensively applied for the isolation of plant-derived polyphenols, including phenolic acids, flavonoids, stilbenes, and tannins. Classical examples include the separation of chlorogenic acids and caffeic acid derivatives from coffee and artichoke, flavonols such as quercetin, kaempferol, and myricetin from onion and grape skins, and resveratrol and its oligomers from grape pomace, typically using silica gel, Sephadex LH-20, or reversed-phase C18 stationary phases with gradient elution systems. Elution chromatography thus represents the cornerstone technique for polyphenol purification prior to spectroscopic or hyphenated chromatographic identification [101,102].

4.6. The Role of Reliable Analysis in Quality Control and Biological Activity Assessment

Reliable analytical characterization of polyphenols is essential for quality control, regulatory compliance, and accurate assessment of biological activity in formulated products. Modern quality control strategies rely on advanced chromatographic and spectrometric techniques, particularly high-performance liquid chromatography coupled with diode-array detection and mass spectrometry (HPLC-DAD-MS and LC-MS/MS) [103]. These techniques provide high sensitivity, selectivity, and structural confirmation of individual polyphenols even in complex matrices. Spectrophotometric assays such as the Folin–Ciocalteu method and antioxidant activity tests (DPPH, ABTS, ORAC) are widely used for rapid screening, although they lack structural specificity. Reliable quantification is crucial not only for standardization but also for correlating polyphenol concentration with biological

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activity. Moreover, method validation parameters such as linearity, accuracy, precision, detection limits, and matrix effects must be rigorously controlled to ensure data reliability. The harmonization of analytical protocols in accordance with international standards (ISO, AOAC, Pharmacopeia) remains a key requirement for industrial application and regulatory acceptance.

4.7. Examples of Translating Laboratory Research into Industrial Applications

Numerous examples demonstrate the successful translation of laboratory-scale polyphenol research into industrial products. Grape polyphenols, particularly resveratrol and proanthocyanidins, are widely utilized in dietary supplements and dermocosmetic products due to their anti-aging and photoprotective properties [103]. Green tea catechins have been incorporated into functional beverages and nutraceutical capsules for cardiovascular protection and metabolic regulation. In the cosmetic industry, polyphenol-rich extracts from berries, pomegranate, and medicinal plants are used as natural antioxidants, preservatives, and skin-brightening agents. Industrial implementation requires not only biological efficacy but also technological feasibility, reproducibility, and economic sustainability. Optimization of extraction processes using green technologies such as ultrasound-assisted extraction, microwave-assisted extraction, and supercritical fluid extraction has significantly improved industrial scalability while reducing environmental impact [8]. Furthermore, encapsulation strategies have enabled the stabilization of polyphenols during processing, storage, and application, thereby facilitating their commercialization in high-value products.

5. Analytical Techniques for Detection and Characterization

5.1. Spectroscopic and Chromatographic-Mass Spectrometric Techniques for Detection and Characterization

The analytical detection and structural characterization of polyphenols rely on a combination of spectroscopic and chromatographic techniques that together provide comprehensive insights into their composition, stability and functional properties. UV-Vis spectroscopy traditionally represents the first analytical step because most phenolic compounds exhibit characteristic absorption bands arising from $\pi \rightarrow \pi^*$and $n \rightarrow \pi^*$transitions within the 240-380 nm region [104]. Flavonoids such as quercetin display two well-defined absorption maxima: Band I, originating from the B-ring and typically occurring between 350 and 380 nm, and Band II, linked to the A-ring and detected between 255 and 270 nm. Stilbenes, including resveratrol, exhibit absorption around 306 nm [105]. Owing to these distinctive spectral signatures, UV-Vis spectroscopy is widely used for rapid evaluation of total phenolic content (TPC), assessment of oxidative processes, and monitoring the stability of polyphenols in food, pharmaceutical, and cosmetic formulations. Nevertheless, its limited selectivity and frequent spectral overlap in complex matrices have driven the integration of UV-Vis detection into more advanced chromatographic platforms. In this context, diode-array detection (DAD) coupled to high-performance liquid chromatography (HPLC) offers a significant improvement by enabling simultaneous acquisition of full absorption spectra for each analyte during elution [106]. This feature markedly enhances the identification capabilities of HPLC-based methods. DAD is particularly effective in differentiating anthocyanins, which absorb strongly between 515 and 540 nm, flavonols with maxima in the 350-370 nm range, and hydroxycinnamic acids absorbing between 310 and 325 nm [107]. Owing to its high spectral resolution and the ability to generate characteristic "UV-Vis fingerprints," HPLC-DAD has become a standard tool in the analysis of wines, grape extracts, fruits, herbal matrices, and cosmetic raw materials. Furthermore, DAD provides valuable information on peak purity, facilitating method validation and improving the detection of co-eluting compounds. Mass spectrometry (MS), especially when coupled with liquid chromatography, is currently the most powerful technique for definitive structural identification of polyphenols. Electrospray ionization (ESI), predominantly operated in negative mode, enables sensitive detection of phenolic acids, flavonoids, stilbenes, tannins, and their conjugates even at trace levels. Tandem mass spectrometry (MS/MS) further enhances analytical specificity by revealing characteristic

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fragmentation pathways, such as neutral losses of glucose (-162 Da), rhamnose (-146 Da), or rutinose (-308 Da), and B-ring fragmentation producing ions at *m*/*z* 151, 179, or 137 [107]. These highly diagnostic fragments make LC-MS/MS the gold standard for profiling dozens to hundreds of polyphenolic compounds in a single analytical run, including structural isomers that cannot be distinguished by UV-Vis or DAD alone. LC-MS/MS has been extensively applied to polyphenol profiling in wine, berries, tea, medicinal plants, honey, and cosmetic formulations, and is instrumental in monitoring their stability and degradation during processing and storage. Nuclear magnetic resonance (NMR) spectroscopy remains the most powerful technique for complete structural elucidation of polyphenols. One-dimensional (ˆ1H, ˆ13C) and two-dimensional experiments (COSY, HSQC, HMBC, NOESY) provide direct information about aromatic substitution patterns, hydroxyl group positions, and glycosidic linkages. NMR is indispensable not only for characterizing newly isolated natural products but also for confirming the structures of stilbenes and flavonoids from grapevine tissues [109], and for analyzing condensed tannins and their oligomers. Quantitative NMR (qNMR) further allows accurate quantification without external calibration standards, which has made it increasingly valuable for the standardization of botanical extracts used in supplements, food, and cosmetics. Modern analytical workflows for polyphenol characterization are increasingly dependent on hyphenated techniques such as LC-MS, LC-MS/MS, and LC-NMR, which integrate chromatographic separation with spectroscopic or mass-based detection. LC-MS provides complementary information on retention times and molecular masses, while LC-MS/MS yields fragmentation spectra that enable precise structural assignments. LC-NMR, although less common due to instrument complexity, offers unparalleled structural detail for compounds that ionize poorly in MS or exist at low concentrations in crude extracts [110]. The combined use of these hyphenated systems has dramatically advanced the discovery of novel polyphenols, determination of degradation pathways, and evaluation of authenticity and quality of botanical materials and derived commercial products.

Multivariate statistical techniques such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) have become essential for interpreting complex datasets generated by LC-MS/MS and HPLC-DAD workflows. PCA is widely applied to uncover natural clustering within samples for example, differentiating grape varieties, botanical species, or processing stages based on their polyphenolic "chemotypes". Meanwhile, PLS-DA enables clear discrimination between predefined sample groups and is frequently used in quality control, detection of adulteration, and authentication of geographical origin [111]. These techniques occupy a central role in modern foodomics, forensic food science, authenticity testing of herbal products, and optimization of extraction processes aimed at maximizing the yield or preserving the stability of polyphenols.

Table 8. Comparative overview of analytical techniques for polyphenol detection and characterization.

Technique	Type of information provided	Key advantages	Limitations	Typical applications
UV-VIs	Absorption maxima; total phenolics	Fast, inexpensive, ideal for TPC/TFC	Low selectivity; spectral overlap	TPC assays; degradation
HPLC-DAD	Retention + full UV-Vis spectra	High resolution: tentative identification	Cannot distinguish all isomers	Profiling anthocyanins and flavonols in wine
LC-MS/MS	Molecular mass + fragmentation	Highest sensitivity and selectivity	High cost Complex data	Identification of flavonoids, stilbenes tannins
NMR	Complete structural information	Definitive structure; qNMR	Lower sensitivity; requires pure sample	Structural elucidation; extract standardization

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PCA/PLS-DA	Chemometric Classification	Powerful pattern recognition	Requires large, quality datasets	Authentication of wine and plant materials

5.2. Hyphenated Techniques: LC-MS, LC-MS/MS, and LC-NMR

The integration of liquid chromatography with spectrometric and spectroscopic techniques has led to the development of hyphenated analytical systems, which represent the most powerful contemporary approach for the characterization of polyphenols. These techniques enable simultaneous separations, identification, and quantification of a large number of phenolic compounds in diverse plant matrices, foods, beverages, and agro-industrial by-products. Over the past decade, technological improvements in sensitivity, resolution, ionization efficiency, and data processing have significantly expanded their application in polyphenol research.

LC-MS has become the standard technique for both quantitative and quantitative profiling of polyphenols. Liquid chromatography separates complex mixtures, while mass spectrometry provides molecular mass, fragmentation patterns, and structural information. During the last ten years, LC-MS has been widely applied across different natural matrices. For instance, Xiong and collaborators (2022) used LC-QTOF-MS to identify more than 120 phenolic compounds in black grape samples, including resveratrol, quercetin, catechins, and oligomeric proanthocyanidins [112]. Similarly, Temerdashev and collaborators (2021) applied Orbitrap-MS to study terrior-specific phenolic profiles in red wine, identifying various flavonoids, stilbenes, and phenolic acids [113]. In tea matrices, Ito and collaborators (2022) used LC-MS to monitor catechin transformation during black tea fermentation, demonstrating changes in EGCG, ECG, and EGC content [114]. Borrás-Linares et al., (2014) successfully applied LC-ESI-MS to Rosmarinus officinalis, identifying 43 phenolic metabolites dominated by quercetin derivatives and rosmarinic acid [115]. LC-MS has also proven effective in the characterization of phenolics from grape pomace; Zhou et al., (2023) reported the presence of resveratrol, kaempferol derivatives, and condensed tannins in grape waste fractions [116]. Collectively, these examples demonstrate that LC-MS is the method of choice for rapid and reliable profiling of polyphenols in complex natural samples.

Tandem mass spectrometry (LC-MS/MS) represents an advanced version of LC-MS and offers exceptional selectivity and sensitivity through multi-stage ion fragmentation. Due to its high specificity and superior matrix-tolerance, LC-MS/MS has become the preferred technique for trace-level quantification of polyphenols in biological, nutritional, and cosmetic samples. Ramalingamet and collaborators (2016) developed a sensitive LC-MS/MS method for monitoring the pharmacokinetics of trans-resveratrol and its glucuronide and sulfate metabolites in human plasma, with a detection limit of only 0.2 ng/mL [117]. Vázquez and collaborators (2021) applied LC-MS/MS to propolis extracts, identifying key flavonoids such as pinocembrin, galangin, and quercetin [118]. In cosmetic formulations, Pieczykolan and collaborators (2022) quantified quercetin, gallic acid, and ferulic acid in antioxidant dermocosmetic products using LC-MS/MS [119]. Furthermore, Mantzouraniet and collaborators (2024) identified 14 phenolic acids in fermented fruit juices using LC-MS/MS, including caffeic, p-coumaric, and sinapinic acid [120]. These examples confirm that LC-MS/MS is indispensable for accurate quantification in complex matrices where polyphenols may appear in ultra-low concentrations.

LC-NMR combines chromatographic separation with structural elucidation based on nuclear magnetic resonance spectroscopy. Although less widespread that MS-based techniques due to lower sensitivity and higher operational complexity, LC-NMR provides unique structural information without requiring reference standards, making it highly valuable in phytochemistry and natural product metabolomics. Over the past decade, LC-NMR has been increasingly used to identify novel or rare polyphenols. Chira and collaborators (2020) employed LC-NMR to elucidate the structure of ellagitannins from oak wood extracts, which could not be unambiguously identified by MS alone [121]. Cheynier and collaborators (2015) successfully used LC-NMR to differentiate cis- and trans-isomers of stilbenes in grape extracts, showcasing the method's capacity to resolve structurally

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similar molecules [110]. Mantzourani and collaborators (2024) applied LC-NMR to pomegranate juice, identifying a complete profile of ellagitannins and flavonols with minimal sample preparation [120]. Recent advances in LC-SPE-NMR protocols, described by Wolfender and collaborators (2019), have further enhanced the ability to isolate and structurally characterize trace-level flavonoids and polymerized tannins [122]. Because of its capability to provide comprehensive structural details, LC-NMR is considered indispensable in cases involving isomers, stereoisomers, or compounds lacking commercial standards.

Table 9. Advantages, limitations, and applications of advanced analytical techniques for polyphenol characterization.

Technique	Main Advantages	Limitations	Typical applications
LC-MS	High sensitivity and selectivity; suitable for profiling large numbers of polyphenols; accurate mass detection (QTOF, Orbitrap)	Possible ion suppression requires skilled optimization of ionization source	Profiling > 120 phenolics in grapes [112]; terrior-specific wine analysis [113]; catechin monitoring in tea [123]; grape pomace metabolomics [116].
LC-MS/MS	Ultra-high sensitivity; ideal for trace quantification; MRM transitions enable excellent matrix tolerance	Requires standards for quantitative methods; limited structural information	Pharmacokinetics of resveratrol metabolites [117]; flavonoids in propolis [118]; phenolics in cosmetics [119]; phenolic acids in fermented juices [120]
LC-NMR	Provides full structural elucidation without standards; ideal for isomers and novel compounds; minimal sample prep	Lower sensitivity; expensive instrumentation; long acquisition time	Identification of ellagitannins in oak [121]; cis/trans stilbene differentiation [110]; polyphenols in pomegranate juice [120]; structural analysis of rare flavonoids [122]

5.3. Application of Multivariate Analysis (PCA and PLS-DA) in the Identification and Classification of Polyphenols

Multivariate data analysis, particularly techniques such as Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA), has become an essential component of contemporary analytical approaches in polyphenol research. These methods enable the simultaneous evaluation of large datasets, which is crucial when studying complex plant extracts, fermented products, or metabolomes rich in phenolic compounds. Over the past decade, the application of multivariate models has increased substantially, especially in combination with LC-MS, LC-MS/MS, and LC-QTOF-MS techniques that generate data of high dimensionality.

PCA is the most widely used unsupervised method for dimensionality reduction and visualization of similarities of differences among samples. In polyphenol analysis, PCA is commonly applied to differentiate plant species, geographical origins, cultivars, and technological treatments. For example, Palade and collaborators (2021) employed LC-MS based PCA to distinguish grape varieties characterized by different levels of stilbenes and flavonols, clearly grouping samples according to terroir-related factors [123]. In tea research, Ramalingam and collaborators (2016) used PCA on LC-MS datasets to successfully classify green, semi-fermented, and black teas based on their catechin content and oxidation products [117]. PCA has also been widely applied for identifying metabolic fingerprints of polyphenols in bee products, botanical extracts, and fermented foods.

Unlike PCA, PLS-DA is a supervised classification method that maximizes separations between predefined groups. PLS-DA is particularly useful for identifying markers responsible for discrimination, which is essential when differences between samples are subtle or occur at trace-level concentrations. Pieczykolan et al., (2022) applied PLS-DA to classify wines according to geographical

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origin using LC-QTOF-MS polyphenol profiles and identified quercetin-3-glucuronide, proanthocyanidins, and p-coumaric acid as key discriminant markers [119]. Similarly, Chen and collaborators (2021) used PLS-DA to differentiate honeys of various botanical origins based on their phenolic composition, identifying rutin, pinocembrin, and caffeic acid phenethyl ester (CAPE) as major contributors to sample separation [50]. In fermented products, PLS-DA has been used to discriminate probiotic fermentations based on catechin and phenolic acid profiles, as demonstrated by Mantzourani and collaborators (2024) [120].

Multivariate analysis plays a critical role in identifying potential polyphenol biomarkers, both in metabolomics and in quality control or authentication of food products. When combined with LC-MS/MS, PCA and PLS-DA enable the identification of specific phenolic compounds that explain differences among samples. Recent studies frequently incorporate Variable importance in Projection (VIP) models. These approaches facilitate the rapid identification of phenolic compounds that contribute most significantly to sample discrimination, making them indispensable tools for metabolic profiling, authenticity testing, and detection of adulteration.

Current trends indicate that the use of PCA and PLS-DA in polyphenol analysis will continue to grow through integration with machine learning and automated pattern-recognition systems. The combination of chemometric techniques with high-resolution mass spectrometry forms the foundation of modern phytochemical research, enabling not only classification and identification but also deeper insights into biosynthetic pathways and phenolic variability influenced by environmental and agroecological factors.

6. Analytical Challenges and Standardization

6.1. Matrix Effects, Degradation, and Stability of Polyphenols

The analysis of polyphenols represents one of the most demanding tasks in modern analytical chemistry due to their structural diversity, high reactivity, and susceptibility to chemical and physical degradation. The complexity of natural matrices – particularly those of plant origin – significantly affects the accuracy and reproducibility of analytical methods. Matrix effects, degradation pathways, and the intrinsic stability of polyphenols are therefore considered major challenges in the standardization of analytical protocols. Over the past decade numerous studies have confirmed that these factors are the primary sources of variability in LC-MS, LC-MS/MS, and spectrophotometric analyses of phenolic compounds.

Matrix effects are among the most dominant issues encountered in polyphenol analysis, especially in samples such as wine, grape extracts, teas, plant oils, honey, and fermented beverages. Natural matrices contain proteins, sugars, organic acids, lipids, and minerals, all of which may induce ion suppression or ion enhancement in LC-MS and LC-MS/MS systems, thereby affecting quantification. Recent studies have shown that polysaccharides and proteins can form noncovalent complexes with flavonoids, reducing extraction efficiency and ionization [124]. In wines, the presence of sulfites and transition metals (e.g., Fe, Cu) can modulate oxidation pathways of polyphenols, while, in plant extracts, high levels of chlorophylls and lipids interfere with chromatographic separations and reduce detection sensitivity. Consequently, modern analytical workflows increasingly rely on matrix-matched calibration, the use of internal standards, and solid-phase extraction (SPE) cleanup to mitigate matrix-induced distortions.

Another major challenge in polyphenol analysis is their chemical instability. Polyphenols are prone to oxidation, isomerization, hydrolysis, and polymerization, and even under optimized conditions they may undergo rapid degradation. For examples, tea catechins readily oxidize in the presence of oxygen and light, forming theaflavins and thearubigins, while resveratrol is highly sensitive to UV exposure and undergoes trans-to-cis isomerization. Flavonoid glycosides such as rutin and quercetin-3-glucoside are susceptible to hydrolysis under acidic conditions. In food systems, phenolic acids may react with proteins and amino acids, forming stable adducts that complicate detection. Degradation can also occur during sample preparation: freezing, drying,

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lyophilization, and extraction with heated solvents can alter phenolic composition, especially in samples rich in oxidases.

The stability of polyphenols depends on multiple factors, including temperature, pH, metal ions, light exposure, and solvent type. Recent studies indicate that storing plant materials at low temperatures with minimal oxygen exposure significantly enhances the stability of flavonoids and stilbenes [125]. It has also been demonstrated that extraction with ethanol or methanol improves stability relative to aqueous systems, while additives such as ascorbic acid and EDTA reduce oxidative degradation. In agro-industrial by-products, where polyphenols are often bound to cellulose and lignin, maintaining stability during extraction poses an even greater challenge.

Standardization of analytical methods for polyphenols requires careful optimization of all steps-sample preparation, extraction, chromatographic separation, detection, and data processing. One of the most persistent issues is the lack of commercially available standards for many phenolic oligomers and tannins, which complicates quantification and inter-laboratory comparability. As a result, considerable efforts are currently directed toward developing relative quantifications strategies, using isotopically labeled internal standards, and validation analytical methods according to international guidelines (e.g., ICH, AOAC). Recent trends highlight an increasing reliance on combined analytical approaches involving LC-MS/MS, high-resolution mass spectrometry (HRMS), and multivariate data analysis, which collectively help compensate for matrix effects and degradation during analysis. This integrated strategy enables not only accurate identification and quantification but also long-term reproducibility, which is essential for food chemistry, pharmaceutical research, and phytochemical studies.

6.2. Lack of Reference Standards and Quantification Challenges

One of the principal limitations in the quantitative analysis of polyphenols is the limited availability of certified reference standards, particularly for structurally complex compounds such as glycosylated flavonoids, oligomeric proanthocyanidins, and various oxidation or conjugation products. This limitation is especially critical in LC–MS and LC–MS/MS analyses, where accurate quantification frequently relies on structurally related compounds used as surrogate standards. Such an approach inevitably introduces systematic bias due to differences in ionization behavior, fragmentation efficiency, and detector response, as highlighted in several studies on polyphenol and anthocyanin analysis [126]. In grape and wine analysis, malvidin-3-*O*-glucoside is commonly employed as a universal calibration standard for anthocyanins. However, it has been clearly demonstrated that the use of this compound as a single reference standard may lead to substantial quantitative deviations when applied to other anthocyanin derivatives. Reported discrepancies of up to 30–50% are primarily attributed to compound-specific differences in electrospray ionization (ESI) efficiencies, which vary significantly among individual anthocyanins depending on their substitution patterns and glycosylation profiles [127]. These findings emphasize the inherent limitations of surrogate-based calibration strategies in LC–MS analysis of structurally diverse anthocyanin profiles. In addition to issues related to reference standard availability, matrix effects represent one of the dominant sources of uncertainty in MS-based quantification of polyphenols. Complex food and herbal matrices contain a wide range of co-eluting endogenous compounds that may cause pronounced signal suppression or enhancement, thereby significantly affecting analytical accuracy. Ion suppression effects of up to 60% have been reported in LC–MS analyses of polyphenols extracted from plant matrices, even when internal standards were applied [128]. These observations demonstrate that internal standardization alone is often insufficient to fully compensate for matrix-induced variability, particularly in highly heterogeneous samples, and highlight the necessity for improved sample cleanup procedures, matrix-matched calibration approaches, and the development of isotopically labeled standards.

As a consequence, quantitative results often lack interlaboratory comparability, which seriously impedes the standardization of analytical methods and their application in regulatory control [129].

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In addition, matrix effects resulting from complex botanical or food matrices continue to be a major barrier to accurate quantification. Ion suppression levels reaching up to 60% have been reported for LC-MS analysis of plant extracts, even when internal standards are used. Similar issues were confirmed in honey, where quantification errors exceeding 40% occurred when matrix effects were not adequately corrected [118]. Such issues severely limit interlaboratory comparability and impede the development of universally applicable quantitative methods.

6.3. Method Validation

Method validation remains essential for ensuring the reliability of analytical techniques used for the determination of polyphenols. Key validation parameters include precision, accuracy, and linearity. Most LC-MS/MS methods report acceptable repeatability at RSD <5% and inter-day precision at RSD < 10%. For example, UHPLC-MS/MS determination of citrus flavonoids achieved RSD values between 1.8% and 6.2%, while recovery experiments demonstrated accuracies of 92-108% [130]. Linearity is generally required over a concentration range from ng/mL to μg/mL with correlation coefficients $R^2 \geq 0.995$. Importantly, the assessment of matrix effects is considered a mandatory part of method validation for MS-based techniques. In the analysis of phenolic compounds in honey, insufficient correction of matrix effects resulted in deviations of more than 40%, highlighting the need for matrix-matched calibration [118]. These observations confirm that method validation must be tailored to each specific sample matrix rather than applied generically.

6.4. Proposed Guidelines for Future Harmonization of Analytical Method

To improve the reproducibility and global comparability of quantitative data, there is a growing need to harmonize analytical methodologies for polyphenol determination. One promising direction is the establishment of centralized MS/MS spectral libraries that include fragmentation patterns, retention times, and UV-Vis profiles of polyphenols, which would greatly facilitate reliable identification of unknown compounds [131].

Regulatory bodies such as the European Pharmacopoeia Commission (2021) emphasize the necessity of standardizing analytical performance criteria and defining reference methods for bioactive compound analysis [132]. Likewise, harmonization of sample preparation protocols – including solvent selection, solid-to-liquid ratio, extraction time, and temperature is crucial for minimizing matrix-dependent variability.

A major component of future harmonization efforts will be interlaboratory validation studies, as promoted in analytical guidelines issued by the European Commission (2022) [133]. These initiatives aim to generate transferable LC-MS/MS workflows that can be adopted across food, nutraceutical, cosmetic, and pharmaceutical analytical laboratories.

7. Applications in Functional Foods, Nutraceuticals, and Dermocosmetics

7.1. Polyphenols as Bioactive Components in Formulations

Polyphenols have attracted considerable scientific and industrial interest as multifunctional bioactive compounds due to their antioxidant, anti-inflammatory, antimicrobial, and photoprotective properties. These characteristics make them particularly suitable for applications in functional foods, nutraceuticals, and dermocosmetic formulations. In functional food systems, polyphenols are commonly incorporated to enhance oxidative stability, improve nutritional value, and confer health-promoting effects beyond basic nutrition [6]. Epidemiological and clinical studies have linked regular intake of polyphenol-rich foods with a reduced risk of chronic diseases, including cardiovascular disorders, metabolic syndrome, and certain types of cancer [25].

In nutraceutical formulations, polyphenols such as flavonoids, phenolic acids, stilbenes, and tannins are widely used either as isolated compounds or as standardized plant extracts. Resveratrol, quercetin, catechins, and curcumin are among the most extensively studied polyphenols due to their demonstrated bioactivities, including modulation of oxidative stress and inflammatory pathways.

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However, their practical applications is often limited by low bioavailability, poor water solubility, and chemical instability. To overcome these limitations, advanced delivery systems such as nanoemulsions, liposomes, biopolymeric nanoparticles, and exosome-based carriers have been increasingly explored to improve the stability and bioaccessiblity of polyphenols in nutraceutical products [134]. In the field of dermocosmetics, polyphenols are recognized as key active ingredients in formulations aimed at skin protection, anti-aging, photoprotection, and skin brightening. Their ability to scavenge reactive oxygen species, inhibit lipid peroxidation, and modulate melanogenesis-related enzymes, such as tyrosinase, underpins their widespread use in topical products [20,135]. Polyphenol-rich extracts from grape, green tea, pomegranate, and various medicinal plants have been shown to protect skin cells against UV-induced damage and premature aging [21]. Recent studies emphasize the growing role of encapsulation strategies, including lipid-based systems and plant-derived vesicles, in enhancing the penetration, stability, and controlled release of polyphenols in dermocosmetic formulations [22]. Overall, the incorporation of polyphenols into functional foods, nutraceuticals, and dermocosmetic products represents a promising and rapidly evolving area of research. Future developments are expected to focus on the optimization of formulation strategies, improvement of bioavailability and skin delivery, and the establishment of standardized analytical and regulatory frameworks to ensure product efficacy, safety, and quality [136].

7.2. Role of Reliable Analysis in Quality Control and Biological Activity

Reliable analytical characterization represents the cornerstone of quality control nd biological activity assessment of products based on plant extracts, nutraceuticals, and dermocosmetic formulations. Due to the pronounced chemical complexity of plant matrices and the significant variability in secondary metabolite composition, precise identification and quantification of bioactive compounds are essential to ensure batch-to-batch consistency and reproducibility of biological effects [122]. Advanced analytical approaches, particularly UHPLC-DAD-MS/MS and LC-HRMS, enable simultaneous targeted and untargeted analysis, thereby reducing the risk of incorrect attribution of biological activity to compounds present only in trace amounts or formed as degradation products [137]. Method validation, including the evaluation of linearity, precision, accuracy, limits of detection, and limits of quantification, has a direct impact on the reliability of correlations between chemical profiles and biological activity. For instance, it has been demonstrated that differences in the quantification of total polyphenols or individual flavonoids can lead to substantial discrepancies in the interpretation of antioxidant or tyrosinase-inhibitory activity of extracts [138]. Studies combining validated chromatographic methods with multivariate data analysis (PCA, PLS-DA) enable the identification of key compounds responsible for biological effects, thereby shifting research from a descriptive to a mechanistic understanding of bioactivity [122].

As an illustrative example, analyses of grape extracts and winery by-products have shown that accurate quantification of resveratrol and its derivatives using LC-MS/MS methods allows a clear correlation between stilbene content and antiradical as well as anti-inflammatory activity, which could not be reliably achieved using spectrophotometric methods alone [139]. These findings clearly confirm that reliable analytical methodologies represent a critical link between chemical composition and biological efficacy.

7.3. Examples of Translation of Laboratory Research into Industry

The translation of laboratory research into industrial practice represents one of the major challenges in the development of functional foods, nutraceuticals, and dermocosmetic products. Although numerous bioactive compounds have demonstrated promising biological effects in in vitro and in vivo models, their industrial application requires raw material standardization, scalable extraction processes, and stable formulations compliant with regulatory requirements [140]. In this context, green and industrially applicable technologies, such as ultrasound-assisted, microwave-assisted, and supercritical CO2 extraction, enable the successful transfer of laboratory protocols to industrial conditions while preserving bioactivity [24].

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One well-documented example of successful translation is the development of standardized polyphenolic extracts (e.g., green tea, grape, or olive extracts), where the clearly defined content of marker compounds is a prerequisite for their commercial use in dietary supplements and cosmetic products [125]. Further advances in translation have been achieved through the development of advanced delivery systems, such as nanoemulsions, liposomes, and exosomes, which significantly improve the stability and bioavailability of poorly water-soluble polyphenols, including curcumin and resveratrol [134].

In dermocosmetics, translation is particularly evident in skin-brightening formulations, where tyrosinase inhibitors identified and validated at the laboratory level (e.g., flavonoids and stilbenes) have been incorporated into stable emulsions with demonstrated efficacy and acceptable safety profiles [135]. These examples highlight that successful translation requires an interdisciplinary approach integrating analytical chemistry, formulation science, biological evaluation, and regulatory frameworks, ultimately enabling the transition from fundamental research to market-relevant, science-based products.

8. Conclusions

Polyphenols represent a chemically diverse and biologically important class of plant secondary metabolites whose efficient extraction, separation, and reliable characterization remain central challenges in food, pharmaceutical, nutraceutical, and cosmetic research. Their broad structural variability, susceptibility to degradation, and strong interactions with complex plant matrices necessitate the use of carefully optimized and often complementary analytical strategies. This review highlights that no single extraction or analytical technique is universally applicable for all classes of polyphenols. Conventional extraction methods such as maceration, Soxhlet extraction, and percolation continue to serve as valuable reference approaches due to their simplicity and reproducibility, while modern green technologies—including ultrasound-assisted, microwave-assisted, pressurized liquid, and supercritical fluid extraction—offer significant advantages in terms of efficiency, selectivity, and environmental sustainability. The increasing use of green solvents such as ethanol, natural deep eutectic solvents, and selected ionic liquids further supports the transition toward safer and more sustainable extraction workflows. Chromatographic separation remains the cornerstone of polyphenol analysis. Partition, adsorption, ion-exchange, size-exclusion, and affinity chromatography each provide distinct selectivity mechanisms that are particularly valuable when dealing with structurally similar compounds or complex natural matrices. The integration of these separation techniques with advanced detection platforms—especially LC–MS, LC–MS/MS, and LC–NMR—has substantially improved the depth, sensitivity, and reliability of polyphenol profiling. These hyphenated systems enable the identification of minor constituents, structural isomers, and degradation products that cannot be resolved using standalone techniques. Despite significant progress, several analytical challenges persist, including matrix effects, limited availability of reference standards, compound instability during sample preparation, and difficulties in method standardization. The growing application of chemometric tools such as PCA and PLS-DA has proven essential for handling large datasets, improving classification, authenticity assessment, and quality control of polyphenol-rich products. Overall, advances in extraction technologies, chromatographic strategies, and hyphenated analytical platforms have greatly expanded our understanding of polyphenol composition and functionality. Future research should focus on further harmonization of analytical protocols, wider adoption of green and scalable technologies, and improved linkage between chemical profiling and biological activity. Such integrated approaches will be crucial for the reliable translation of polyphenol research from laboratory studies to industrial applications and regulatory frameworks.

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Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ASE	Accelerated Solvent Extraction
CE	Capillary Electrophoresis
CO_2	Carbon Dioxide
COSY	Correlation Spectroscopy
DAD	Diode-Array Detection
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EC	Epicatechin
ECG	Epicatechin Gallate
EGCG	Epigallocatechin Gallate
ESI	Electrospray Ionization
GC	Gas Chromatography
GRAS	Generally Recognized As Safe
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HPLC	High-Performance Liquid Chromatography
HRMS	High-Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
ILs	Ionic Liquids
ISO	International Organization for Standardization
LC	Liquid Chromatography
LC–HRMS	Liquid Chromatography–High-Resolution Mass Spectrometry
LC–MS	Liquid Chromatography–Mass Spectrometry
LC–MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LC–NMR	Liquid Chromatography–Nuclear Magnetic Resonance
MAE	Microwave-Assisted Extraction
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NaDES	Natural Deep Eutectic Solvents
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
ORAC	Oxygen Radical Absorbance Capacity
PCA	Principal Component Analysis
PLE	Pressurized Liquid Extraction
PLS-DA	Partial Least Squares Discriminant Analysis
qNMR	Quantitative Nuclear Magnetic Resonance
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
$scCO_2$	Supercritical Carbon Dioxide

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SEC	Size-Exclusion Chromatography
SFE	Supercritical Fluid Extraction
TFC	Total Flavonoid Content
UAE	Ultrasound-Assisted Extraction
UHPLC	Ultra-High-Performance Liquid Chromatography
UV–Vis	Ultraviolet–Visible Spectroscopy

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