Advances in extraction and analysis of phenolic compounds from plant materials

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[ABSTRACT] Phenolic compounds, the most abundant secondary metabolites in plants, have received more and more attention in recent years because of their distinct bioactivities. This review summarizes different types of phenolic compounds and their extraction and analytical methods used in the recent reports, involving 59 phenolic compounds from 52 kinds of plants. The extraction methods include solid–liquid extraction, ultrasound-assisted extractions, microwave-assisted extractions, supercritical fluid extraction, and other methods. The analysis methods include spectrophotometry, gas chromatography, liquid chromatography, thin-layer chromatography, capillary electrophoresis, and near-infrared spectroscopy. After illustrating the specific conditions of the analytical methods, the advantages and disadvantages of each method are also summarized, pointing out their respective suitability. This review provides valuable reference for identification and/or quantification of phenolic compounds from natural products.

[KEY WORDS] Phenolic compounds; Flavonoid; Extraction; Quantification; Liquid chromatography; Gas chromatography

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Introduction

Phenolic compounds (PCs), the most abundant secondary metabolites in plants, are found ubiquitously in our life. Many medicinal herbs have been found to be abundant in PCs. These plants include *Boehmeria nivea* (L.) Gaudich. [1], *Salvia miltiorrhiza* Bge. [2-3], *Ginkgo biloba* L. [4], *Acanthopanax senticosus* (Rupr. et Maxim.) Harms [5], *Myristica fragrans* Houtt. [6], and *Cimicifuga foetida* L. [7]. Furthermore, fruits [8-10], vegetables [11], spices [12], and cereals [13] are also common sources of PCs, especially polyphenols, in our daily diets [14]. PCs possess a common chemical structure comprising an aromatic ring with one or more hydroxyl substituents that can be divided into several classes, and the main groups of PCs include flavonoids, phenolic acids, tannins, stilbenes, and lignans [15].

In recent years, with the increasing recognition for their medicinal values, PCs have been found to help reduce the risk of many chronic diseases [16]. As numerous studies reported, PCs exert various effects such as antioxidant [17], anti-microbial [18], anti-carcinogenic [19], anti-inflammatory [20-21], and estrogen-related [22] prevention of cardiovascular diseases [23-25], cancers [1], diabetes [26], and diseases associated with oxidative stress [27]. For example, vanillic acid, a kind of phenolic acid obtained from *Angelica sinensis* (Oliv.) Diels (Apiaceae), exhibits reducing activity in acetylcholinesterase (AChE), tumor necrosis factor (TNF-α), and corticosterone with improved antioxidants that contribute to neuroprotection [28]. Resveratrol, a kind of stilbene, may contribute to the prevention of retinal pigment epithelium degeneration induced by oxidative stress [29]. Therefore, these recently discovered properties of PCs have been exploited in the development of cosmetics [30], nutraceuticals [8], or functional foods [31].

In the research or development of PCs, exploring qualitative or quantitative approaches to analyzing these bioactive substances should be prioritized in abundant different natural sources, which contribute to developing rapid, sensitive, and reliable methods. Many different methods have been explored or improved in the past years. General approaches allow the

quantitation of a global estimation of PC content (e.g., "total flavonoids" or "total phenolic"), which are mainly achieved by spectrophotometry methods. However, more specific analyses are based on the identification of individual phenolic classes, typically by high-performance liquid chromatography (HPLC) or gas chromatography (GC) and their detection by sensitive detectors, such as mass spectrometry (MS) [32-34]. Some advanced techniques are also applied to quantify PCs, including capillary electrophoresis [35] and near-infrared (NIR) spectroscopy. Before the analysis processes, extraction methods should also be selected and optimized along with the corresponding analytical techniques, including the used sol-

vents, the sources, and the properties of the compound itself.

Therefore, developing an optimized and proper method for extraction and quantification of PCs is essential for achieving higher accuracy in results. To the best of our knowledge, although some articles have been published on relevant fields, the studies are relatively outdated and scattered. This review summarizes some aspects of different types of PCs, their extraction procedures, and related analytical methods for quantification in the last 5 years. The main advantages as well as the limitation of each method are compared to profile useful information for the determination of PCs in plant materials. The structure diagram of the methods is shown in Fig. 1.
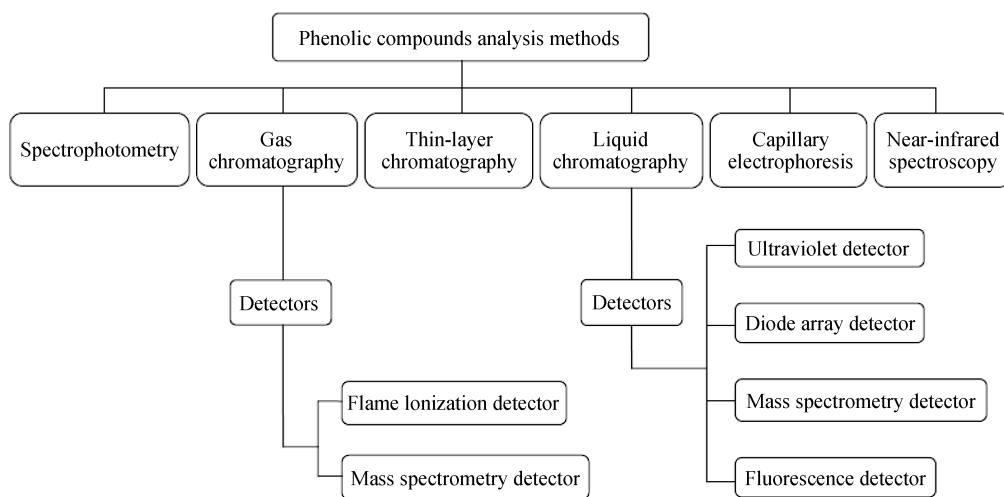

Fig. 1   Structure diagram of methods for analysis of PCs

Types of PCs

Flavonoids

Flavonoids constitute the largest group of PCs from plants. To date, more than 8000 PCs, including over 4000 flavonoids, have been identified, and the number continues growing [36]. The flavonoid consists of 15 carbon atoms arranged in three rings (C6–C3–C6) labeled as A, B, and C, respectively; A and B are two aromatic rings, and C is a three-carbon bridge, usually in the form of a heterocyclic ring. On the basis of saturation degree and C-ring substituents, flavonoids are divided into six subgroups, including flavonols, flavones, flavanones, isoflavones, flavanonols, and anthocyanins. For example, rutin and quercetin exist in herbs, such as *Flos sophorae* Immaturus, *Crateagus pinnatifida* Bunge, *Hypericum japonicum* Thunb, and *Folium* Mori [37]. Epicatechin, a flavonoid isolated from the Mexican medicinal plant *Geranium mexicanum* HBK, could affect virulence properties of human pathogen [39]. Another major flavonoid, kaempferol, which is obtained from *Kalanchoe blossfeldiana* Poelln., has anti-herpes potential [41].

Isoflavones, a subclass of flavonoids, hold structural similarity to estrogens. Some biological functions are attributed to their structural similarities to *β*-estradiol [22], and

therefore isoflavones are sometimes referred to as "phytoestrogens", which are especially abundant in soybeans. Studies have shown that they can be used to prevent some prevalent diseases, such as atherosclerosis [42] and cancer [43], and ameliorate muscle wasting [44].

Anthocyanins, the most important group of water-soluble vacuolar pigments, appear as red, blue, or purple and occur in all plant tissues, including flowers, stems, leaves, roots, and fruits. These substances are abundant in berry fruits (such as black currant, raspberry, and blueberry). Anthocyanins may have anti-inflammatory and antimicrobial effects [45]. Furthermore, anthocyanins (cyanidin-3-*O*-beta-glucoside chloride or cyanidin chloride) exert protective effects in diabetic nephropathy by inhibiting the liver X receptor alpha pathway-induced inflammatory response [46].

Phenolic acids

Phenolic acids belong to a major class of PCs in plants and present in free and bound forms. Phenolic acids can be divided into two subgroups: hydroxybenzoic acid (HBA) and hydroxycinnamic acid (HCA). HBAs are based on a C6–C1 structure and include *p*-hydroxybenzoic acid, protocatechuic, vanillic, gallic, and syringic acids. However, HCAs are aromatic compounds with a three-carbon side chain (C6–C3), including coumaric, caffeic, ferulic, and sinapic acids [47].

Studies have shown that phenolic acids have various biological functions. For instance, caffeic acid can dramatically increase the proportion of the mucin-degrading bacterium *Akkermansia* in colitis by dextran sulfate sodium, and thus ameliorate colonic pathology and inflammation [48]. Gallic acid can reduce neural damage and brain amyloid neuropathology and improve cognitive function by scavenging free radicals and inhibiting *β*-amyloid oligomerization [49] and salvianolic acid B, a polyphenol from *S. miltiorrhiza* Bge., may prevent meningococcal infections by inhibiting meningococcal binding and may thus affect the number of nasopharyngeal carriers of *Neisseria meningitides* [50]. Vanillic acid, obtained naturally from the plant *A. sinensis* (Oliv.) Diels (Apiaceae), can be an effective therapeutic agent for treating neurodegenerative disorders [28].

Tannins

Tannins are a kind of compounds with a relatively high molecular weight, which comprise another major group of PCs in the plant kingdom. Tannins can be divided into two subgroups, including hydrolysable tannins (HT) [51] and condensed tannins (CT). Given the products of hydrolysis reaction, most HTs are subdivided to gallotannins and ellagitannins. CTs are oligomers or polymers of flavan-3-ol monomers, most frequently linked by an interflavan carbon bond. Tannins have been reported to contain potential metal ion chelators [52], protein-precipitating agents [53], and biological antioxidants [54] with various biological functions. However, predicting an accurate mechanism in biological systems is difficult because of enormous structural variations. In the future, we must focus on the study of structure and function relationships so that biological functions can be predicted.

Stilbenes

Stilbenes are minor compounds composed of a 1, 2-diphenylethylene nucleus with some hydroxyls. Studies have shown that the number of stilbenes is low, and the main representative compound is resveratrol, which exists in both cis and trans isomeric forms [55]. Resveratrol has been detected in many plant species, including grapes [56], almonds [57], *Gnetum parvifolium* [58], and *Polygonum cuspidatum* Sieb. et Zucc. [59]. Given antioxidant and anti-inflammatory properties, stilbenes hold a potential for preventing different diseases, such as cancer and those associated with oxidative stress [29].

Lignans

Lignans are produced by the oxidative dimerization of two phenyl-propane units and can be found in a wide variety of plants, such as *Arctium lappa* L. [60] and *Schisandra chinensis* (Turcz.) Baill. [61], oil seeds [62], and cereal grains [63]. In particular, the flax seed is a rich source of lignans. In recent years, focus of research has been on their potential applications of antioxidant, anti-inflammatory, and cancer chemopreventive properties [64].

Extractions Methods

Extraction procedure is a primary step in identifying and/or quantifying the process of chemical compounds. Numerous reports focus on the extraction and analysis of PCs from plant materials, including herbs, fruits, and vegetables. Many conventional means can be used to extract PCs, such as solid–liquid extraction (SLE) and heated reflux extraction. In addition, a number of advanced methods, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), or high hydrostatic pressure extraction (HHPE), are also applied in the extraction of PCs from plant materials. Different extraction methods of PCs were illustrated and compared in Table 1, with applicability description.

Table 1 Comparison of different extraction methods of PCs

Extraction method	Advantages	Applicable to	Disadvantages	Inapplicable to	Example	Ref
SLE	simple and easy to operate; wide adaptability.	general used	the need to use substantial amounts of hazardous organic reagents; long extraction time; low efficiency	/	catechins	[65]
UAE	simple and easy to operate; efficient; economical; wide adaptability.	general used	unsuitable for industrial production	/	caffeic acid isoferulic acid ferulic acid	[7]
MAE	consumes less extraction solvent and extraction time; increases the content of extracted antioxidants	thermally stable PCs	degradation and oxidation will occur under such conditions	PCs with more -OH, thermolabile PCs	caffeic acid 5-*O*-caffeoylquinic acid quercetin-7-*O*-glucoside 4, 5-Dicaffeoylquinic acid 3, 5-Dicaffeoylquinic acid	[66]
SFE	increase safety and selectivity; avoid sample oxidization in the presence of air.	non-polar PCs	unsuitable for the extraction of polar PCs; high requirement of capital investments	polar PCs	gallic acid protocatechuic acid *p*-hydroxybenzoic acid	[67]
ASE	require small amounts of solvents; provides faster extraction processes.	thermally stable PCs	the need of high temperature and pressure	thermolabile PCs	quercetin-3-*O*-glucuronide flavonol-3-*O*-glycosides	[68]
HHPE	efficient; consumes less extraction solvent and extraction time.	general used	the need of high pressure and expensive equipment	/	polyphenol	[69]

Solid–liquid extraction (SLE)

SLE is the simplest and the most commonly used method for extracting PCs from various plants. In general, SLE of PCs consists of direct extraction of fresh or freeze-dried plant materials with different solvents, such as methanol, ethanol, acetone, or the aqueous phase of solvent mixtures [65, 70], and then requires additional operation, such as subsequent column chromatography or solid-phase extraction, to remove these unwanted substances. Tomaz *et al.* have developed a SLE method to extract flavonoids (anthocyanins, flavonols, and flavan-3-ols) from red grape skins [71]. Hadaruga *et al.* have extracted *Ficaria verna* Huds. flowers and leaves by semi-continuous SLE [72]. Several parameters may influence the PC yield, including extraction time, temperature, solvent-to-sample ratio, solvent type and polarity, and the number of repeat extractions of the sample [73]. These differences could be attributed to the different properties of PCs in plants.

In addition, different hydrolysis methods are usually used in the extraction of PCs. Lu *et al.* [74] have developed a new method for the analysis of 20 phenolic compounds, where the samples are prepared by hydrochloric acid hydrolysis. It is found that hydrochloric acid hydrolysis is of similar effectiveness to, but much cheaper than, the enzymatic method. In addition, to obtain the best yield of PCs, researchers have also added formic acid and sulfuric acid [75-76]. Furthermore, researchers can obtain best results with the addition of an alkaline catalyst (sodium hydroxide) [77] and enzyme (cellulase, tannase, beta-glucosidase, and pectinases) [78]. A novel ethanol-alkaline extraction method is designed for the extraction and purification of aglycone isoflavones from soybean. The cellulase is used instead of traditional beta-glucosidase to hydrolyze the glycoside isoflavones resulting in excellent conversion efficiency [79]. Addition of a catalyst is an effective method for gaining the best yield of PCs extraction; however, special attentions should be paid to the amount of acid to be added, since excess acid or alkaline could hydrolyze labile substances during extraction process.

SLE is simple and easy-operating, but several disadvantages exist, including: (1) the need to use substantial amounts of hazardous organic reagents; (2) long extraction time; and (3) low efficiency. Therefore, some advanced techniques are required to overcome these problems.

Ultrasound-assisted extractions (UAE)

UAE is a useful and economic technology because expensive instruments are not required for its use. Sonication is the production of sound waves that create cavitation bubbles near the sample tissue, which disrupt plant cell walls, thereby releasing cell contents [80]. Extracting efficiency is influenced not only by sonication time, temperature, and ultrasonic wave frequency, but also by the property of solvent and sample. Dashi *et al.* [81] have developed a UAE method for extracting the PCs (caffeic acid, tannic acid, quercetin, transferulic acid, and rosmarinic acid) from *Stachys parviflora* L., which applies a 4-min treatment time, 74.5% high-intensity (UP 200Ht,

Dr. Hielscher GmbH, Germany) and 74.2% methanol as the optimal extracting conditions, with the yield of total phenolic content as 20.89% and total flavonoid as 6.22%. Furthermore, UAE has been also developed for the extraction of PCs from the leaves of native specimens of *Myrcia amazonica* DC. [82], *Cimicifugae foetida* L. [7], *Urtica fissa* E. Pritz [83], and *Polygonum cuspidatum* Sieb. et Zucc. [84]. In addition, Gonzales *et al.* [85] have combined alkaline hydrolysis and UAE for the release of nonextractable phenolics from *Brassica oleracea* var. botrytis Waste to enhance efficiency. In summary, UAE is a simple, efficient, and economical method in laboratory.

Microwave-assisted extraction (MAE)

MAE is a mature technique used for PC extraction from various plant materials. MAE utilizes the direct effect of microwave energy to facilitate partition analytes from the sample into the solvent. The major physical parameters important for MAE include microwave power, extraction time, solubility, dielectric constant, and solvent property. Solvent property is an important factor because solvents with high dielectric constants can absorb more microwave energy; methanol, ethanol, and water are commonly used to extract PCs from plants [66]. Dahmoune *et al.* [86] have developed MAE to extract PCs from *Myrtus communis* L. instead of UAE and conventional solvent extractions. They have also observed that the antioxidant activities of tannins and total flavonoids in MAE extracts are higher than that of the products extracted by other extraction methods [86]. These findings suggest that extraction of bioactive phytochemicals from plant materials using MAE method consumes less extraction solvent and extraction time and increases the content of extracted antioxidants. In addition, MAE has been successfully used for the extraction of PCs from other plant materials, such as *Glycyrrhiza uralensis* Fisch [87], *Eclipta prostrata* L. [66], *Urtica fissa* E. Pritz, and *Rosmarinus officinalis* [88]. However, PCs with higher number of hydroxyl-type substituents [89] and those that are sensitive to temperature [90] may not be suitable for extraction by MAE because of the degradation and oxidation that occur under such conditions.

Supercritical fluid extraction (SFE)

SFE is an environmentally friendly extraction technique, which can be an alternative efficient extraction method for PCs. The most commonly used supercritical fluid is supercritical CO_2. A number of other supercritical fluids, such as ethane, butane, pentane, nitrous oxide, ammonia, trifluoromethane, and water are also used. Ghafoor *et al.* have developed SFE to extract the PCs from grape seeds, and the grape seeds extracts are also analyzed for HBAs, including gallic acid, protocatechuic acid, and *p*-hydroxybenzoic acid [67]. Compared with other methods, SFE may consume less toxic organic reagents and extraction times, increase safety and selectivity, and avoid sample oxidization in the presence of air [11]. However, CO_2 is non-polar and hence unsuitable for the extraction of polar PCs. Moreover, the major weakness of this method is the high requirement of capital investment.

Accelerated solvent extraction (ASE) is an advanced technique that extracts PCs from plant materials under high temperature and pressure. High temperature and pressure contribute to rapid solvent penetration into plant cells and prevent PC degradation. Compared with conventional methods, such an approach provides faster extraction processes that require small amounts of solvents [91]. Monschein *et al.* [68] have applied ASE to extract flavonoids from *Epilobium angustifolium* L.

High hydrostatic pressure extraction (HHPE) is another recent technique that can be applied to extract PCs from plants. The high hydrostatic pressure promotes rapid solvent penetration into plant cells and causes leakage of cell components. Lee *et al.* have used HHPE to extract polyphenol from *Panax ginseng*. C. A. Mey [69]. However, the main disadvantage of the method is the need for expensive equipment.

Analytical Methods

PC quantification depends on different parameters, such as the chemical nature of compounds, extraction method used, particle size, standard selection, and interfering substances and impurities. With the advancement of analytical science, numerous methods have been used for quantifying PCs from plant materials, such as spectrophotometry, HPLC, GC, and their combinations.

Spectrophotometry

Spectrophotometry is a simple and fast technique for quantifying PCs from plant materials, which is mainly based on different principles for measuring the various structures present in the PCs. The Folin–Ciocalteu assay has been widely used to detect PCs in plants for many years. This assay is based on a chemical reduction involving reagents containing tungsten and molybdenum [92]. The Folin–Ciocalteu method is a modified method of the Folin–Denis assay, which slightly changes the composition of reagent used. In general, the flavonoid content is often measured with spectrophotometry [93-94]. Pezzini *et al.* have developed spectrophotometric method to determine the total flavonoid content in leaves of *Ocimum basilicum* (herbal material) [95]. In addition, total phenolic quantification [96] and the condensed tannin content [97] can also be estimated by spectrophotometry. Spectroscopy is the common technique used for quantifying different classes of PCs because of its simplicity and low cost. However, the main disadvantage of the spectrophotometric assays is that such assays estimate the PC content without a separation process and accurate quantitative results of individual PCs. Moreover, the reagents used in these methods (Folin–Ciocalteu method) do not react specifically with only PCs, but also with other substances, such as ascorbic acid.

Gas chromatography (GC)

GC is a useful technique utilized for the separation, identification, and quantification of some PCs in plants, such as tannins, flavonoids, and anthocyanins [98-99]. The derivatization and volatility of PCs are the main components detected by GC. GC combined with a flame ionization detector [64] is the most common method for measuring PCs [100]. Vaiciulyte *et al.* [101] have applied GC-FID to detect carvacrol obtained from *Thymus pulegioides* L.

In recent years, GC coupled with mass spectroscopy (MS) detector has become widespread in measuring complex compounds because of its high selectivity and sensitivity in quantitation. For example, the low-molar-mass fraction of hydrophilic extracts in Norway spruce knotwood, which are mainly lignans, has been characterized by GC–MS [102]. GC–MS has also been used in specific lignan fingerprint profiles and quantitation of characteristic compounds in *Schisandra chinensis* (Turcz.) Baill. [103]. However, the main concern with this method is the low volatility of PCs. PCs are usually transformed into more volatile derivatives by methylation or converted into trimethylsilyl derivatives [104]. GC offers better capability for compound identification, however, there are still many challenges remaining in the course of analyzing phenolic compounds, when detecting compounds have low volatility, since their analysis requires transformation in derivatives to enhance volatility.

Liquid chromatography (LC)

HPLC is the most used technique for the separation and detection of PCs. Some factors affect HPLC analysis of PCs, such as column types, applied detectors, mobile phase, and the properties of the tested compounds. Some application cases of LC and hyphenated conditions in analysis of PCs are presented in Table 2.

HPLC–UV detector (HPLC–UVD)

The most used HPLC detection system for measuring PCs is based on UV absorption. Zhang *et al.* have developed a HPLC–UVD procedure for determining multiple flavonoids and phenolics from *A. senticosus* (Rupr. et. Maxim.) Harms [5]. Al-Rimawi *et al.* have developed and validated a HPLC–UVD method for determining eight phenolic acids (gallic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and sinapic acid) in date palms [105].

HPLC–diode array detector (HPLC-DAD)

HPLC coupled with DAD is another common method for analyzing the PCs in plants [51, 106]. Generally, the principles underlying the DAD and UVD are the same; however, some differences exist in other aspects. For example, DADs are used to simultaneously measure multichannel absorption wavelengths. Meanwhile, UVDs are used to measure single-absorption wavelength in known samples. Silva Siqueira *et al.* have developed HPLC–DAD to determine the PCs (chlorogenic acid, caffeic acid, rutin, and isoquercitrin) from *Spondias tuberosa* [106]. Majid *et al.* have developed HPLC–DAD to determine the PCs (rutin, catechin, caffeic acid, and myricetin) in *Euphorbia dracunculoides* [107]. Some phenol substituting compounds are considered as highly toxic compounds

Table 2 Application of LC and hyphenated conditions in analysis of PCs

Source	Stationary phase	Mobile phase	Detector	Ref
Vaccinium myrtillus L.(leaves) *Vaccinium vitis-idaea* L. (leaves)	XB-C$_{18}$ (3.6 μm, 150 mm × 4.60 mm)	A: formic acid–water B: formic acid–acetonitrile	DAD-ELS-MS/MS	[100]
Psidium guajava L. (leaves)	Poroshell 120, SB-C$_{18}$ (3.0 mm × 100 mm, 2.7μm)	A: water–acetic acid B: acetonitrile	DAD-ESI-QTOF-MS	[10]
Salvia miltiorrhiza Bge (leaves and stems)	RP -C$_{18}$ (150 mm × 3 mm, 5μm)	A: formic acid–water B: acetonitrile	DAD-MS	[3]
Eclipta prostrata L. (overground part)	MS-C$_{18}$ (50 mm × 2.1 mm, 2.1μm)	A: methanol B: aqueous formic acid	DAD–ESI–MS/MS	[66]
Spondias tuberosa (leaves)	Luna C$_{18}$ (4.6 mm × 250 mm, 5μm)	A: acetonitrile B: water–acetic acid	DAD/MS	[106]
Centella asiatica (L.) Urb (overground part)	RP-C$_{18}$ (250 mm × 4.6 mm, 5μm)	A: phosphoric acid–water B: acetonitrile	UV	[51]
Euphorbia dracunculoides (overground part)	Sorbex RX C$_8$ (25 mL capacity, 5μm)	A: acetonitrile–methanol–water–acetic acid B: acetonitrile–methanol–acetic acid	DAD	[107]
Helianthus tuberosus L. (leaves)	Eclipse XDB-C$_{18}$ (250 mm × 4.6 mm, 5μm)	A: methanol B: formic acid–water	MS	[110]
Haplophyllum tuberculatum (overground part)	Nova-Pak C$_{18}$ (300 mm × 3.9 mm, 4 μm).	A: water–acetic acid B: water–acetonitrile–acetic acid	MS	[27]
Schisandra chinensis (Turcz.) Baill (leaves and fruits)	Zorbax SB-C$_{18}$ (100 mm × 3.0 mm , 3.5 μm)	A: methanol B: acetic acid–water	UV-MS	[109]
Agrimonia pilosa Ledeb	Welch ultimate XB-C$_{18}$ (4.6 mm × 250 mm, 5 μm).	A: phosphate–water B: acetonitrile	UV	[113]
Malaysian cocoa powder	RP-C$_{18}$ (250 mm × 4 mm, 5μm)	A: water–trifluoroacetic acid B: acetonitrile-trifluoroacetic acid	DAD/ESI-MS/MS	[17]
Lycium barbarum L. (leaves) *Lyciu chinense* Mill. (leaves)	Zorbax SB-C$_{18}$ (100 mm × 3.0 mm, 3.5 μm)	A: methanol B: acetic acid–water	UV-MS	[18]

usually found in environmental water samples, especially in urban wastewaters. Villar–Navarro *et al.* have detected 2,4-dichlorophenol, 2,5-dichlorophenol, 2,6-dichlorophenol, pentachlorophenol, 2,4-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitriphenol tert-butylphenol, and sec-butylphenol in water samples by HPLC-DAD [108]. In summary, UVD and DAD are applicative to the tested compounds with a suitable chromophore.

HPLC-MS

PCs can be analyzed by LC combined with tandem MS. HPLC assisted by MS detection is an advance analytical technique that exhibits high sensitivity and selectivity. This approach can measure structural information about unknown compounds from crude or partially purified samples of natural sources [109]. Recently, numerous studies on PC analyses have been focused on the assessment of methods that involve different couplings between LC and MS. Chen *et al.* have analyzed the PCs in Jerusalem artichoke (*Helianthus tuberosus* L.) responding to salt stress by LC/tandem MS [110]. Eissa *et al.* have developed the polyphenols (benzoic acids, cinnamic acids, flavones, and flavanones) from the aerial parts of *Mentha longifolia* L. by HPLC–MS [111]. In recent years, MS is usually used for the analysis of PCs because of its high sensitivity and selectivity; in addition, it could provide structural information about unknown compounds.

HPLC-fluorescence detector (HPLC-FLD)

HPLC coupled with FLD is also used to analyze PCs. Curcumin and bisdemethoxycurcumin from *Curcuma longa* L.

extracts [112] could be detected by HPLC-FLD. Godoy–Caballero *et al.* have applied LC-DAD-FLD to analyze some important PCs, such as gallic acid, caffeic acid, luteolin, syringic acid, gentisic acid, vanillic acid, and ferulic acid in virgin olive oil samples. In addition, HPLC-FLD has also been used to measure some phenol substituting compounds, such as tert-butylphenol and sec-butylphenol [108]. HPLC coupled with FLD can be used to detect the substance which emits fluorescent or emit fluorescent after proper derivations, although it doesn't obtain a suitable fluorescent group.

ELSE analytical methods

Thin-layer chromatography (TLC) is a chromatographic technique with relatively low cost. Through this process PCs in crude plant extracts can be separated and detected for multiple substances on the same TLC plate within a short analysis time. Males, Z *et al.* [114] have used TLC to quantify the flavonoids and chlorogenic acid in the leaves of *Arbutus unedo* L. The determination and quantification of PCs can be carried out by TLC, if one wants to detect multiple substances in a short analysis time.

In addition, capillary electrophoresis [35] is an advanced analysis technique for measuring PCs in plants. CE is a high-resolution technique conducted with a solution of ions in a narrow capillary column, which is especially suitable for separating and quantifying those polar and charged compounds with low to medium molecular weight. For example, rutin contained in germinated buckwheat can be analyzed by CE [115]. Boiteux *et al.* have developed a CE method for de-

termining catechin, naringenin, cinnamic acid, syringic acid, chlorogenic acid, apigenin, vanillic acid, luteolin, quercetin, and caffeic acid in *Thymus mongolicus* Ronn, *Origanum vulgare*, and *Matricaria recutita* [9]. The largest disadvantage of CE is that it lacks the ability to discriminate compounds with close charge-to-mass ratios. Therefore, one can add organic solvents to enhance the separation capability of CE.

Moreover, near-infrared (NIR) spectroscopy is an advanced, accurate, and non-destructive analytical technique for analyzing PCs from natural sources. The NIR region of the electromagnetic spectrum covers the visible and infrared regions with a known wavelength range of 780–2 500 nm. Krahmer *et al.* have applied NIR spectroscopy to predict the PC contents or organic acids, as well as individual substances, such as epicatechin or lactic acid [116].

Conclusion

This review presented some advanced techniques utilized in the extraction and analysis of PCs. The advanced extraction methods include SLE, UAE, MAE, SFE, ASE, and HHPE. Among these procedures, SLE is the simplest method and has wide adaptability, but it requires substantial chemical reagents and has relatively low extraction efficiency. UAE is a simple, efficient, and economical method that is usually used in laboratory. Compared with other methods, MAE consumes less extraction solvents and extraction time, and its efficiency is relatively high, but may not be suitable for extraction of some thermolabile PCs. SFE is an environmentally friendly technique, but it requires high capital investment and may be unsuitable for extracting polar PCs. Among analytical methods, HPLC has major advantages, especially when coupled with highly sensitive sophisticated detectors (e.g., MS). The diversification of the detectors enhances the sensitivity and specificity of analysis of the tested compounds. With proper derivatization method, GC is more suitable for PCs when the PC volatility is increased. Moreover, TLC and CE can also be developed for analyzing PCs. TLC provides more information about the samples than the other methods, whereas CE is adequate for charged low- to medium-molecular-weight compounds. Based on the full consideration on the property of the tested compounds and actual analysis conditions, appropriate analytical methods should be selected. This review offers valuable reference for the identification and/or quantification of PCs from natural products.

Non-standard abbreviations

Abbreviation	Full name
PCs	Phenolic compounds
AChE	Acetylcholinesterase
MG	Methyl gallate
NIR	Near infrared spectroscopy
ALPL	Alkaline phosphatase
HBA	Hydroxybenzoic acid
HCA	Hydroxycinnamic acid
HT	Hydrolysable tannins
CT	Condensed tannins
SLE	Solid-liquid extraction
ASE	Accelerated solvent extraction
HHPE	High hydrostatic pressure extraction
FID	Flame ionization detector
UVD	Ultraviolet detector
DAD	Diode array detector
FLD	Fluorescence detector

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