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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

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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 solvents, 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.

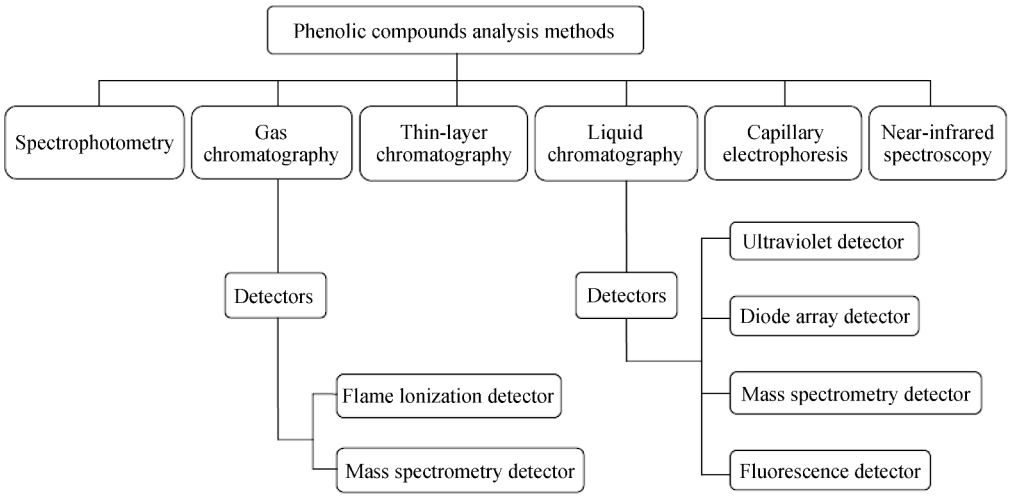

Fig. 1 Structure diagram of methods for analysis of PCs

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].