Chapter

Extraction Techniques of Phenolic Compounds from Plants

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Abstract

Phenolic derivatives are one of the most important compounds that were found in secondary metabolites in plants. According to their various applications in agriculture, food, chemical and pharmaceutical industries, interests in reviewing different procedures of extraction of these compounds from plants have increased. In this chapter, we would like to have an overview on the extraction procedures that have been used in isolating phenolic compounds from plants until this time, including liquid-liquid extraction (LLE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE). In the following, advantages and disadvantages of these techniques and methods will be discussed and explained. In addition, in the last part of this chapter, various methods for purification and identification of phenolic compounds will be presented.

Keywords: plants, phenolic compounds, bioactive compounds, extraction techniques, identification techniques

1. Introduction

Plants are rich and valuable resources of bioactive phenolic. They can be utilized in various fields such as antioxidant, antimicrobial, anti-inflammatory, antitumor, antiviral, analgesic and antipyretic [1, 2]. Hence, they have attracted the attention of many health professionals and many organizations and health care systems increasingly recommend the daily consumption of fruits and vegetables [3, 4].

Phenolics are one of the major and diverse group of active compounds in the plants which have at least one aromatic ring and one or more hydroxyl groups in their structures [5, 6]. They can be divided in two categories. First category consists of soluble compounds such as flavonoids, quinones, phenylpropanoids which can be discovered in plant cell vacuole and the second category consists of insoluble compounds such as lignins, condensed tannins and hydroxyl cinnamic acid which are detected in cell-wall bound [7]. All of these groups are involved in many processes in plants. Various classifications of phenolic compounds based on various subgroups and structures have been listed in **Table 1**. Due to importance and worthiness of phenolic compounds on human health, many researches have been so far done in order to synthesizing various compounds like natural compounds using synthetic sequence procedures [8–11].

Based on various structures of phenolics, they have different physical and chemical characteristics which are very significant and emphasize on the extraction

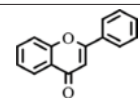
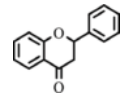
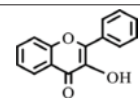
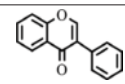
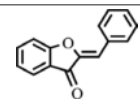
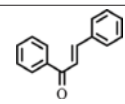
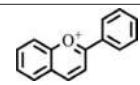
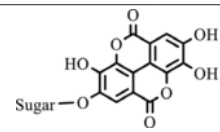
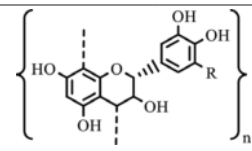
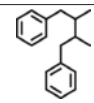
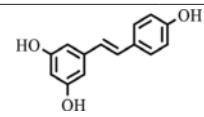
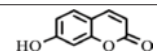
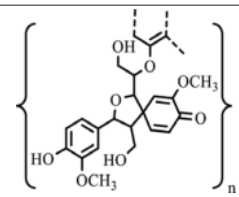
Basic group	Subgroup	Structure
Phenolic acids	Hydroxybenzoic acid derivatives	
	Hydroxycinnamic acid derivatives	
Flavonoids	Flavones	
	Flavanones	
	Flavonols	
	Isoflavones	
	Aurones	
	Chalcones	
	Anthocyanins	
Tannins	Hydrolysable tannin	
	Condensed tannins	
Miscellaneous group	Lignans	
	Resveratrol	
	Coumarins	
	Lignins	

Table 1.
Classification of phenolic compounds based on various subgroups and structures.

processes [12]. Therefore having knowledge about various methods of extraction, identification and quantification of phenolic compounds are essential and useful. In this chapter various techniques for extractions of phenolic compounds with their advantage and disadvantage will be investigated. Finally in the last section of this chapter, various methods have been discussed to identify and evaluate their quality.

2. Liquid-liquid extraction (LLE)

Scientists using various solvents have studied and investigated the extraction of phenolic compounds of different parts of plants such as leaves and seeds. Based on simplicity and cheapness of this extraction method, various polarities of solvents and under different temperature and pH conditions, they have been able to extract various combinations of phenolics from plants [13].

Plants contain various amounts of phenolic compounds which have simple and complicated structures. As there is a possibility of interaction between these compounds and other compounds in plants like carbohydrates and proteins, therefore it will be difficult to find an appropriate method for the extractions of all phenolic compounds [14]. In the liquid-liquid extraction (LLE) methods, various phenolic compounds are extracted and then an extra step for their purification is required.

There are three methods for extractions of phenolic compounds using LLE method which include Soxhlet extraction, maceration and hydro distillation methods. The effective parameters in these extraction methods are the type and polarity of solvents and their ratio, time and temperature of extraction and moreover chemical composition and physical characteristics of the samples [15].

Kerrouri et al. investigated the total content of phenolic compounds, flavonoids and condensed tannins with different solvents with different polarities using Soxhlet extractor and maceration methods [1]. Based on the results obtained in this paper, the various groups of phenolic compounds were extracted by solvents with different polarity so that hexane, ethyl acetate and methanol were used as best solvents for polyphenols, flavonoids and tannins respectively. Therefore they evaluated their antioxidant activities by their reaction with 1,1-diphenyl-2-picryl-hydrazyl radical. The results demonstrated that the antioxidants contained in the *Anethum graveolens* extracts were able to giving hydrogen to a free radical to eliminate potential damage. In another work, the separation of phenolic compounds from oil mixtures by three imidazolium-based dicationic ionic liquids was investigated by Wu et al. [16]. 1,4-bis[N-(N′-methylimidazolium)]butane dibromide demonstrated the maximum phenol removal yield of 96.6% within 5 min. The reusability of dicationic ionic liquids structures were investigated and indicated they were stable after four cycles without a decrease in phenol removal efficiency.

In the liquid-liquid extraction, usually 1–30 g of phenolic compounds within 6–24 h can be extracted. The advantages of this method are that, they have simple extraction procedures and various phenolic compounds can be extracted by organic solvents with different polarities but the disadvantages of this procedure are that they suffer from high solvent consumption, long extraction times and low extraction yields, exposure risk to organic vapors and degradation of target compounds during the extraction method [15]. The problems with these extraction methods are caused to creation and development of alternative techniques that in the following of this chapter will be explained.

3. Ultrasound-assisted extraction (UAE)

Ultrasound waves occur at frequencies between 20 kHz and 10 MHz which pass through solid, liquid and gas and also human is not able to hear it. In this extraction method, cavitation bubbles are created near the sample tissue then they break the cell wall and therefore cell content is released [17].

Ultrasound waves are applied and used by two probe and bath systems for extraction of phenolic compounds from plants. Beside of the inherent parameters of ultrasonic devices (such as amplitude, frequency and wavelength), their power and intensity also have a lot of effects on extraction process that they need to be optimized. Design and shape of reactor and also shape of probe can effect on extraction process [18].

Comparing to traditional methods, ultrasound-assisted extraction (UAE) method has been considered according to its simplicity, easy handling, low cost, high efficiency, lower organic solvent consumption and reduced extraction time. It can be used as a simple and reliable procedure in extensive range of organic solvents for various phenolic compounds in large-scale level and industry [19].

When the extraction process is underway on large-scale, temperature, time and type of solvent can be effective not only on the extraction efficiency but also on extraction compositions and they must pay a lot of attention. Therefore precise study of these parameters in order to obtain the best extraction efficiency is so important at industry. Although it is worth to mention that, high efficiency is not just the only main issue of the extraction process but the less renewable energy sources consumption is also very important in large-scale level and industry [15].

Sun et al. reported the effects of acoustic energy density (6.8–47.4 W/L) and temperature (20–50°C) on the extraction yields of total phenolics by ultrasound-assisted extraction from grape marc [20]. They used 50% aqueous ethanol as the solvent. They mentioned that, the initial extraction rate and final extraction yield with the increase of acoustic energy density and temperature increase higher due to higher diffusion coefficients. Moreover, the comparison between ultrasound technology and two conventional extraction methods showed that ultrasound is a competitive and effective extraction technology for extracting phenolic compounds from grape marc. The extraction yield of selected phenolic acids and flavonoids from *Equisetum arvense* L. herb carried out by Oniszczuk et al. [21]. Different extraction methods like oxhlet extraction, ultrasound-assisted extraction (USAE), and accelerated solvent extraction (ASE) were used in this work. They have mentioned that, ultrasound assisted extraction at 60°C in three cycles for 30 min, with 80% aqueous solution of methanol was a more effective and accurate method than other methods for isolation of selected phenolic acids, and flavonoids from *E. arvense* L. herb. In another research plan by Palma et al., they investigated various parameters such as temperature, output amplitude, duty cycle, the quantity of sample and the total extraction time for extraction phenolic compounds from grapes and compared optimum conditions with traditional extraction techniques [22]. The result showed that ultrasound assisted extraction was able to extract phenolic compounds in higher yield and much shorter extraction time, 6 min instead of 60 min. Also the extraction of phenolic compounds from Syrah grape skin was done by the Tonon et al. [23]. They optimized ultrasound power, citric acid concentration and solid to liquid ratio for this extraction method. Under optimum conditions, 59% of the quantified phenolic compounds with only 3 min of processing were extracted which compared to conventional extraction, ultrasound was considered a suitable method based on facilitating release of phenolic compounds from matrix. In the same way, Zardo et al. have reported extraction of phenolic compounds from sunflower seed cake [24]. The temperature and ethanol concentration showed the

highest effect on the total phenolic compounds extraction from sunflower cake. The results showed that high amounts of phenolics compounds were obtained in the first minute of extraction and longer time did not effect on amount of extraction. Also in the other research plan Row et al. performed extraction of phenolic compounds from Laminaria japonica Aresch with three kinds of 1-alkyl-3-methylimidazolium with different cations and anions [25]. The results showed that, the characteristics of both anions and cations have remarkable effects on the extraction efficiency. Comparing the results of extraction under optimal conditions with conventional solvent showed highest extraction efficiency within the shortest extraction time. Sheng et al. evaluated antioxidant activities of phenolic compounds extracted from *Terminalia chebula* Retz. fruits by different extraction methods like UAE and LLE [26]. The results showed that, the antioxidant activities of phenolic compounds extracted by UAE under optimized condition were stronger

Sample	Phenolic compound	Solvent	Time (min)	Temperature (°C)	mg compound/g dry sample	Ref.
Grape marc	—	50% aqueous ethanol	80	50	1.57 mg/g	[20]
Equisetum arvense L. herb	Chlorogenic acid (CGA), caffeic acid (CA), ferulic acid (FA), isoquercitrin (IQ), 5-glucoside luteolin (5GL)	80% aqueous methanol	30	60	0.5631 mg_{CGA}/g, 0.4739 mg_{CA}/g, 0.2120 mg_{FA}/g, 0.2629 mg_{IQ}/g, 0.2485 mg_{5GL}/g	[21]
Grapes	Phenolic compounds (PC), tannins (TA), anthocyanins (AN)	50% aqueous ethanol	6	10	13 mg_{PC}/g, 7.2 mg_{TA}/g, 1.5 mg_{AN}/g	[22]
Syrah grape skin	Phenolic compounds	50% aqueous ethanol	4–10	20–80	11,732 mg/100 g	[23, 31]
Areca husk	Gallic acid	41% aqueous ethanol	38	53	15.37 mg/g	[32]
Sunflower seed	Gallic acid (GA), chlorogenic acid (CA)	43% aqueous ethanol	3	70	1867.3 mg_{GA}/100 g, 1645.8 mg_{CA}/100 g	[24]
Olive leaves	oleuropein (OLE), verbascoside (VER), luteolin-40-O-glucoside (L4OG)	80% aqueous ethanol	1	60	13.386 mg_{OLE}/g, 0.363 mg_{VER}/g, 0.527 mg_{L4OG}/g	[33]
Flax seeds	Lignan (LI), flavonol (FL), hydroxycinnamic acids (HA)	water with 0.2 N of sodium hydroxide	60	25	24.07 mg_{LI}/g, 6.84 mg_{FL}/g, 11.25 mg_{HA}/g	[34]
Brosimum alicastrum leaf	Total phenolic content (TPC), total monomeric anthocyanin (TMA)	80% aqueous methanol	20–10	28	45.18 mg $_{GAE}$/g, 15.16 mg_{CyE}/100 g	[35]
Grapefruit leaves	Gallic acid	10.80% aqueous ethanol	58.52	30.37	19.04 mg/g	[36]

Table 2.
Optimized condition for extraction of phenolic compounds from plants using UAE.

than LLE method. Recently, Wang et al. reported extraction of hemicellulose and phenolic compounds from bamboo bast fiber powder [18]. The nature of extracted phenolic compounds depends on the used solvent and they reported extraction of hemicellulose and phenolic compounds using ultrasound without adding harmful solvents. The results demonstrated that, the efficiency of extractions increased by 2.6-fold for ultrasound treated samples without solvent in comparison with extractions that use water. In fact, ultrasound can be as used as a green technology for the extraction of bamboo components without adding solvent.

Based on various literatures about ultrasound-assisted extraction method, phenolic compounds can be extracted within 10–60 min and compared to the LLE method, which is easy to be handled, inexpensive, safe and reproducible and can be simultaneously used for a wide range of samples [27, 28]. Further in UAE method, less solvent is required which is a great importance for environment and economical point of views and makes the industry more inclined to use this method in large-scale (**Table 2**). These properties represent that ultrasound-assisted extraction method is beneficial alternative instead of LLE method for extraction of phenolic compounds from different part of plants [29, 30].

It should be mentioned that in this method, an additional filtration step is required. Also as other extraction methods, degradation of compounds is possible at high frequencies.

4. Microwave-assisted extraction (MAE)

Microwave is electromagnetic radiations with frequencies in the range of 30–300 MHz. They generate heat due to the induction of molecular motions, which causes the cell wall to be ruptured and active substance in cell is released.

Dipolar solvent molecules such as water with higher dielectric constant than non-polar solvents can absorb high energy therefore they increase the speed and efficiency of extraction phenolic compounds [37].

There are two microwave-assisted extraction (MAE) systems for extraction of phenolic compounds which are closed vessel and open vessel. In the closed vessel system the extraction happens in the high pressure and temperature while in the open vessel system, the extraction of phenolic compound occurs in atmospheric pressure [38].

Until now, many literatures have been reported about applications of microwave technology for extraction of phenolic compounds from plants. Reducing time and cost, high efficiency and lower organic solvents consumption are advantages of this method than traditional methods. Also another important benefit of this extraction procedure is simultaneous extractions of several substances in a short time [15].

Proestos and Komaitis et al. carried out extraction of phenolic substances from aromatic plants with different solvents by microwave assisted extraction [39]. Comparing these results with conventional (reflux) extraction showed that, extraction by microwave assisted extraction could perform in shorter extraction time, less solvent usage and better extraction yield. Also Memon et al. investigated extraction, identification and antioxidative properties of flavonoids of leaves and flowers of *Cassia angustifolia* [40]. The extraction of flavonoids performed by five various methods including microwave extraction, Soxhlet extraction, sonication extraction, marinated extraction and reflux condensation extraction. Comparison of total flavonoids content with various extraction methods showed that, microwave extraction method is the best option and can extract 28.15–26.3 mg/g in the flowers and leaves, respectively. The advantages of this method were that it was very easy, robust and the extraction was carried out in the 9 min, which is the shortest time

required in comparison with other methods. Therefore quantity and antioxidant activity for this extraction method was found to be the more efficient for extracting more numbers of flavonoids.

Guolin et al. carried out microwave-assisted extraction of pectin from lemon peels using ionic liquid as alternative solvent [41]. Under the optimal conditions and 9.6 min duration time, the extraction efficiency of pectin was 24.68%, which is much higher than the efficiency from the conventional heating reflux extraction under 2 h duration. According to this result, ionic liquid solutions could be considered as an effective solvent in microwave-assisted extraction of pectin from lemon peels. Also Liu et al. evaluated extraction and determination of taxifolin *Larix gmelinii* by ionic liquid-based microwave-assisted extraction method (ILMAE) [31]. They investigated different kinds of 1-alkyl-3-methylimidazolium ionic liquids with different kinds of cations and anions. According to the results, both anions and cations parts of ionic liquids could affect to the extraction yields and optimal solvent was 1-butyl-3-methylimidazolium bromide for the taxifolin extraction. Comparing this method with traditional method including water stirring extraction (WSE), water reflux extraction (WRE), and maceration showed that, the ILMAE method could be done in higher extraction yield, lower energy and time consumption. In the following, under optimum extraction conditions, taxifolin was stable and there was no degradation on it.

Antioxidant activity and inhibitory effect of phenolic compounds extracted by microwave assisted extraction method, are higher and more efficient than

Sample	Phenolic compound	Solvent	Time (min)	Temperature (°C)	mg compound/g dry sample	Ref.
Leaves and flowers of *Cassia angustifolia*	Flavonoids	70% aqueous ethanol	9	50	28.15 $mg_{flowers}$/g, 26.30 mg_{leaves}/g	[40]
Medicinal plants	Flavonoids (FV) Phenolic acids (PA)	50% aqueous ethanol	3–5	70	0.84 g_{FV}/100 g, 2.01 g_{PA}/100 g	[2]
Green coffee beans	Chlorogenic acids	Water	5	50	23.93 mg/g	[47]
Morus nigra leaves	Gallic acid	50% aqueous ethanol	28	120	19.7 mg/g	[48]
Olive Leaf	Phenolic compounds	80% aqueous methanol	6	80	—	[49]
Cherry laurel fruit	Chlorogenic acid (CA), vanillic acid (VA)	Methanol	12–16	65	35.21 mg_{CA}/g 1.19 mg_{VA}/g	[50]
Cherry laurel leaf	Chlorogenic acid (CA), luteolin 7-glucoside (L7G)	Methanol	12–30	65	30.44 mg_{CA}/g 22.78 mg_{L7G}/g	[50]
Larix gmelinii	Taxifolin	$[C_4mim]Br$	14	—	18.47 mg/g	[31]
Psidium guajava Linn leaves	Gallic acid (GA), ellagic acid (EA), quercetin (Q)	Ionic liquid	10	70	0.507 mg_{GA}/g 2.387 mg_{EA}/g 0.540 mg_{Q}/g	[51]
Smilax china tubers	*trans*-Resveratrol (*t*-R), quercetin (Q)	Ionic liquid	10	60	0.531 mgt_{-R}/g 0.189 mg_{Q}/g	[51]

Table 3.
Optimized condition for extraction of phenolic compounds from plants using MAE.

LLE method because a shorter time is required in microwave assisted extraction. According to this fact, Yuan et al. reported the extraction of phenolic compounds at 110°C for 15 min from four economic brown macroalgae species using MAE. These extracted compounds had a higher antioxidant activity and total phenolic content for all algae species in comparison with those obtained by conventional extraction at room temperature for 4 h [42]. In another report from Alara et al., phenolic compounds were extracted from Vernoniacinerea leaves by microwave assisted extraction and they had antioxidant and anti-diabetic activity more than LLE method [43].

Overall, effective parameters of this extraction procedure are solvent, pressure, temperature, nature of matrix and power of device. In microwave-assisted extraction method compared to the LLE, it is easy to handle and less solvent is required [44–46]. Further, this method can reduce the extraction time and increase the extraction yield. The dark point of this method is that, solvent must absorb microwave power and also there is a risk of explosion in this method (**Table 3**).

5. Supercritical fluid extraction (SFE)

In this method, the solvent is at a temperature and pressure above its critical point and there is no surface tension in it. Therefore it simultaneously has the properties of liquid and gas which can be much efficient for extraction of phenolic compounds from plants. Low viscosities and high diffusivities of supercritical fluids enable them to extract the various phenolic compounds in less time with higher efficiency. So it's a good alternative instead of using liquid-liquid extraction methods [52]. A distinguished property of supercritical fluid extraction is that, the density of supercritical fluid can be easily changed based on various temperatures and pressures. Also in constant temperature, solubility in a supercritical fluid is directly related to its density so that by increasing pressure, its solubility tends to increase. Hence, these properties can be used to separate phenolic compounds with various selectivity [53].

Carbon dioxide is known as a stable, nontoxic, environmentally safe, cheap, and selective extraction solvent for supercritical fluid extraction. Based on these outstanding properties, many literatures have been published that carbon dioxide has been used as solvent for extraction of various phenolic compounds [54, 55]. Murga et al. reported selective extraction of some phenolic compounds from grape seeds using carbon dioxide and alcohol under supercritical conditions [52]. However, carbon dioxide is a non-polar solvent and to achieve higher efficiency and selectivity in phenolic compounds extraction, they adjusted the polarity of carbon dioxide by adding methanol and ethanol on it. Various phenolic compounds under optimized conditions were extracted which are mentioned in **Table 4**. Vatai et al. investigated extraction of phenolic compounds from elder berry by supercritical carbon dioxide [54]. They optimized pressure, temperature and carbon dioxide/ethanol mixture ratio for this extraction method and compared the results with liquid-liquid extraction method. The results demonstrated that, supercritical extraction with carbon dioxide can improve the extraction of total phenols from elder berry and hence, it is a good alternative procedure to replace instead of organic solvents.

The antioxidant capacities of phenolic compounds extracted by supercritical fluid extraction are much higher than LLE method. Recently, Pereira et al. assessed antioxidant activity of bioactive compounds which were extracted from myrtle leaves and berries by supercritical fluid extraction method [56]. They used three different methods for investigation of antioxidant activity of these compounds and the results demonstrated that, the extracted phenolic compounds by supercritical fluid extraction methods have a higher antioxidant activity than LLE method. Also, Tan et al. reported that, antioxidant capacity of virgin avocado oil extracted

Sample	Phenolic compound	Pressure (bar)	Time (min)	Temperature (°C)	mg compound/g dry sample	Ref.
Defatted milled grape seeds	gallic acid (GA), protocatechuic acid (PCA), monogalloyl glucose (MG), protocatechualdehyde (PRA), (+)catechin(C), (−) epicatechin (E)	300	180	40	0.034 mg_{GA}/g 0.015 mg_{PCA}/g 0.002 mg_{MG}/g 0.003 mg_{PRA}/g 0.058 mg_{C}/g 0.038 mg_{E}/g	[52]
Elder Berry	Total phenols, quercetin (Q), *t*-resveratrol (*t*-R)	150–300	—	40	74.6 mg_{GA}/g 152 mg_{Q}/100 g 21.0 mgt_{R}/100 g	[54]
Vitis labrusca B	Total phenols (TPC), anthocyanins (A)	157–161	—	45–46	2.156 mg_{TPC}/100 mL 1.176 mg_{A}/mL	[59]
Olive leaves	Phenolic compounds	334	140	100	4.2 mg/g	[53]
Agricultural by-product (wheat straw)	Lignin derived bioactive compounds e.g., tricin and catechins	140	—	35	—	[60]
Eugenia uniflora	Phenolic compounds	400	360	60	240.5 mg_{GAE}/g	[61]
Guaraná seeds	Phenolic compounds (pyrogallol)	100	40	40	105.76 mg/g	[55]
Sasa palmate	Gluconic acid, β-siosterol, α-amyrin acetate	200	—	95	7.31 mg/g	[62]

Table 4.
Optimized conditions for extraction of phenolic compounds from plants using SFE.

using supercritical fluid extraction was higher than UAE and LLE methods [57]. Moreover, avocado oil extracted using supercritical fluid extraction had two to four times bigger levels of α- and γ-tocopherols than LLE and UAE.

In conclusion, the supercritical fluid extraction method is rapid, inexpensive to run and selective, so that even a small available amount of phenolic compounds in plants can be extracted using this method. Also, this method requires a small amount of solvent or no solvent and thermally labile compounds will be stable during this extraction method [58].

6. The purification and identification of phenolic compounds

A very large number of literatures have been published about separation, purification and identification of phenolic compounds in plants. According to the importance of this matter, many improvements have been achieved in this field. These protocols and procedures can be used for identifying and isolating the compounds with a high precision. Since various parts of plants have phenolic compounds with different structures and chemical properties, so that various protocols and spectroscopic techniques such as UV-visible spectroscopy, near-infrared reflectance spectroscopy, nuclear magnetic resonance and high performance liquid chromatography (HPLC) have to be used for their purifications and identifications.

6.1 UV-visible spectroscopy

This spectroscopic method can be used for measuring absorption spectroscopy in the ultraviolet-visible spectral region which relates to electronic transitions in

molecules. Based on aromatic structure of phenolic compounds, they are powerful chromophores in the UV rang so that flavones, phenolic acids and total anthocyanins have absorptions spectrums in the region 320, 360, and 520 nm respectively [63]. The advantages of this spectroscopic method are less time consuming, inexpensive and reproducible. The limitations of identification of this method get raised when multiple combinations have partially overlapping peaks. In order to solve this problem, the method must be used with other spectroscopic methods such as mass spectroscopy or HPLC, simultaneously [64].

6.2 Infrared spectroscopy

This spectroscopic method uses a wide range of wavelength from 780 nm to 1 mm. According to its wavelength, it can be divided to three regions; the near-, mid- and far-infrared. These electromagnetic wavelengths cause vibrational changes in the molecules. Therefore, it is an efficient method for identification of molecules and especially their functional groups, so that various functional groups such as single bond, double bond, triple bond, carboxyl, hydroxyl and amino groups have diverse vibrational frequencies. In fact, with this spectroscopy method, we cannot draw the structure of a chemical compounds and we can just understand the nature of the functional groups in the structures [63]. Abbas et al. investigated the mid-infrared spectra of 36 standard phenolic compounds. The survey of the MIR spectra at the 1755–1400 cm^{-1} and 1000–870 cm^{-1} regions for these compounds showed that, MIR method was able to distinguish between flavonoids and phenolic acids families. They finally mentioned that, in order to have a better distinguishment between various families of phenolic compounds it is necessary to investigate a larger number of samples [65].

6.3 Nuclear magnetic resonance

It is a technique based on the measurement of electromagnetic radiation in the radio waves area. Unlike the infrared and UV-visible spectroscopic methods which electrons are involved in absorption process, this technique is related to the magnetic properties of atomic nuclei. Commonly ^{1}H NMR and ^{13}C NMR are used to study the chemical structure of materials but it can be also applicable for any kind of nuclei possessing spin. Using this spectroscopy method, in addition to identifying chemical compounds, and accurate information on the structure, dynamics, reaction state and chemical environment of molecules can be provided too [66]. Many articles have been published in this regard demonstrating that ^{1}H NMR spectroscopy can be an effective and a useful method for identifying and analyzing of phenolic compounds in plants [63, 67]. Furthermore, this method is fast, quantitative and non-destructive.

6.4 High performance liquid chromatography

This technique is one of the most efficient and impressive chromatographic method that has widely been used for separation, identification and quantification of phenolic compounds. In this method, using the mobile phase at high pressure, sample mixture will be separated from each other on stationary phase. Generally, for the separation and quantification of phenolic compounds, the HPLC is preferred than gas chromatography (GC). Various factors can effect to the efficiency of HPLC which include sample purification, mobile phase, column types, and detectors [33]. Various literatures have been published regarding purifying and quantifying phenolic compounds using HPLC technique [49, 68]. Normally, various phenolic

compounds can be separated using normal phase C18 or reversed phase (RP-C18) column in the presence of different solvents with different polarities. Also it should be mentioned that pH mobile phase should be stabilized in the range of 2–4 to avoid the ionization of phenolic compounds. Therefore, aqueous acidified mobile phase has to be used during purification and quantification of phenolic compounds [23].

In the HPLC technique, the selection of detector plays a significant role in the identification process and depends on the properties of the phenolic compounds. High performance liquid chromatography-mass spectrometry, (HPLC-MS), is highly selective and has a low limit of detection and shorter analysis time [40]. Today, this method is being extensively used because of it is enormous benefits. La Torre-Carbot et al. investigated characterization and quantification of 20 phenolic compounds in olive oils through a combination of the HPLC-DAD and HPLC-MS/MS methods [69]. This method was fast, precise and sensitive. Also this method required a low solvent and sample consumption. HPLC-UV can be used for phenolic compounds that have UV spectrum and thus, selection of wavelength is the most important point for this method so that the solvent and extra available compounds should not have absorption in this wavelength [51]. HPLC with fluorescence detection. (HPLC-FLD), is an efficient method for identification and quantification of phenolic compounds that have fluorescence spectrum. The fluorescence detector sensitivity is 10–1000 times higher than UV detector [70]. HPLC with electrochemical detection is based on redox reaction of phenolic compounds. This method is fast, low cost, precise and low limits of detection of phenolic compounds [71]. Cantalapiedra et al. have used HPLC for separating vanillin, eugenol, thymol and carvacrol using amperometric and coulometric detectors [72]. They reported that, the coulometric detection has a low limit of detection in the range between 0.81 and 3.1 μg/L and is very competitive and sufficient for quality control of phenolic compounds in comparison with other methods, such as GC-MS which are expensive and complicated.

7. Conclusions

In summary, phenolic compounds extracted from plants, have different applications as antioxidant, antimicrobial, anti-inflammatory, antitumor, antiviral, analgesic and antipyretic. Therefore, they have this ability to improve human health. In this chapter, various extraction methods of phenolic compounds from plants were presented and their advantages and disadvantages were explained. According to the structure and extraction source of these compounds, different extraction methods can be recommended. Suggested methods need to be simple and rapid with a high. Also an important point for selecting an extraction method is that, it should be environmentally friendly. In addition, in the last part of this chapter various methods for purification and identification of phenolic compounds were presented with their advantages and disadvantages.

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