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Exploring Natural Phenolic Compounds

Recent Progress and Practical Applications

Edited by Irene Gouvinhas and Ana Novo Barros

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IntechOpen Book Series

Biochemistry

Volume 66

Aims and Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of the life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, coenzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation, etc. More recently, biochemistry embraced the 'big data' omics systems. Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today, however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., the bovine rumen.

This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Meet the Series Editor

Ana Maria Carmona-Ribeiro has been a full professor of Biochemistry at the University of São Paulo (USP), Brazil, since 2001. She founded the Biocolloids Laboratory at USP in 1993, and since then she has been developing novel important assemblies aimed at drug and vaccine delivery. Her background in Physics, Chemistry, Biology, and Pharmaceutics has been useful for the development of biomolecular assemblies with potential for novel biomedical applications.

Meet the Volume Editors

Irene Gouvinhas is an auxiliary researcher at the Centre for Research and Technology in Agro-Environmental and Biological Sciences (CITAB) and the Associate Laboratory Inov4Agro – Institute for Innovation, Capacity Building, and Sustainability of Agri-Food Production. She has been actively involved in several national and international R&D projects, including coordinating roles. Her work focuses on Agrarian and Natural Sciences, with a particular emphasis on the valorization of major agro-industrial by-products and waste streams for the recovery of high-value compounds for diverse industrial applications. She is the author of 50 papers indexed in the Journal Citation Reports (JCR), has supervised students at various academic levels, and regularly contributes to academic life, including the organization of scientific events and serving on examination committees.

Ana Novo Barros is an Associate Professor with Habilitation from the University of Trás-os-Montes and Alto Douro. She is responsible for the Phytochemicals Laboratory and serves as the Coordinator of the Agri-Food Quality Group. Her research targets are mainly in the identification, separation, and recovery of functional molecules from different natural products, as well as their implementation as ingredients and bioactive compounds in food, pharma and cosmetic industries, with an ultimate goal to extract high added-value molecules and re-use them in the circular economy and industrial symbiosis concepts. In recent years, she has received several prestigious awards and distinctions. She has published more than 140 scientific documents, including research articles, reviews, and editorials, in the highest-impact-factor journals in the field of Food Science and Technology (37 h-index, SCOPUS, May 2025). She has registered 9 national and international patents. She has international collaborations and is a principal investigator (PI) and member of several funded research projects with diverse typologies (over the last 5 years). She is the coordinator for the European project WASTELESS, Topic ID: HORIZON-CL6-2022-FARM2FORK-01-08.

Contents

Preface

Phenolic compounds are increasingly recognized for their diverse roles in health promotion, food preservation, and sustainable innovation. Their extraction from natural sources and subsequent application in various industries have been the focus of growing scientific interest.

This book brings together recent developments in the field, combining fundamental concepts with emerging techniques and practical applications. Topics range from theoretical principles of phenolic extraction to advanced strategies, including nanotechnology and the valorization of agro-industrial by-products.

By integrating different perspectives, the volume aims to support researchers and professionals looking to deepen their understanding of natural phenolics and explore new opportunities for their use.

We are grateful to all the authors for their contributions and to the reviewers who ensured the quality of the chapters. We also acknowledge the support of the IntechOpen editorial team throughout the development of this volume.

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Section 1

Extraction, Characterization, and Theoretical Insights

Chapter 1

Perspective Chapter: Theoretical Foundations of the Extraction Process

Shokhista Usmonovna Mirzaeva and
Bakhodir Timurovich Muxamadiev

Abstract

The goal of the research is to create an environmentally friendly, resource-efficient technology for the production of ingredients from plant raw materials using liquefied gases. To a certain extent, this scientific research serves to fulfill the tasks specified in the decrees and decisions adopted in this direction and other normative documents: No. 63 of 27.01.2018 "Cultivation and industrialization of licorice and other medicinal plants in the Republic of Uzbekistan on the measures to further develop the processing method." Decision of the Cabinet of Ministers of the Republic of Uzbekistan, President of the Republic of Uzbekistan dated January 7, 2022 No. PF-60 "Development Strategy of New Uzbekistan for 2022-2026 and the announced Decree of the President of the Republic of Uzbekistan. "On measures to further accelerate work on systematic support of families and women" PF dated March 7, 2022 – The competition organized together with the Family and Women's Committee under the Ministry of Poverty Alleviation and Employment within the framework of priority tasks defined in Clause 5.2 of Decree No. 87. In addition, experimental studies of the process of extracting ingredients from plant raw materials using diluted carbon dioxide in the example of namatak, chamomile grapes, pumpkin and melon seeds, fruit seeds, pomegranate peel, as well as beech root (other local raw materials) will be held. Experiments were conducted to determine the influence of the main factors on the extraction of vegetable oils (extracts) based on the developed plan of experiments, mathematical models of the process were obtained as a result of mathematical-statistical processing of the experimental results, rationalization of the influencing factors parameters (pressure in the extractor, process temperature and duration) values are determined.

Keywords: extraction, biologically active substances, temperature, pressure, extraction time, ingredients from plant raw materials

1. Introduction

Solid–liquid extraction is the extraction of one or more components from a complex solid by selective solubility. Extraction – extraction from a complex solid or liquid

substance of one or more of its components using a solvent with selective solubility ([1–3], pp. 520–550; [4], p. 256; [5], p. 256; [6], p. 186; [7], p. 146).

The extraction process includes the following processes: diffusion, dialysis, dissolution, desorption, osmosis, and mechanical washing.

Diffusion is the process of gradual mutual penetration of substances that border on each other.

The extraction process goes as follows:

a. The solvent diffuses into particles of raw materials, gets to the surface of the cell through intercellular pathways, and penetrates into the cell through the cell membrane.

b. In the interior of the cell, following desorption, biologically active substances are dissolved in a solvent.

c. Due to unequal concentrations, dialysis occurs – the transfer of ingredients from the cell through the cell wall.

d. As a result of dialysis, a fixed diffusion part is formed on the outside of the plant material. The diffusion part is the countermeasure for extracting the ingredients, as inhibits the extraction of ingredients from the material.

e. After passing through the diffusion part, the ingredients spread throughout the entire volume of the solvent according to the laws of free convective diffusion.

Extraction is based on experimental provided and production skills, which generalizes the concept of the influence of various factors on the extraction process ([8], pp. 11–34; [9], p. 416; [10], pp. 894–913).

The significance of extraction is explained by its ability to provide an almost exhaustive separation of dissolved components at low temperatures, which is the key to obtaining high-quality extracted substances ([3], pp. 520–550; [8], pp. 11–34). Traditional physical processing methods in oil and fat production are crushing, pressing, mixing, settling, filtering and thermal influence. Electrophysical and acoustic and other methods are considered non-traditional ([8], pp. 11–34; [11], pp. 40–42; [12], pp. 322–327).

Solvent extraction is the most widely used method in plant extraction. The principle is based on the transfer of a compound from a sample to an organic solvent ([13], pp. 169–198). These are very important operations in many industries such as chemical, biochemical, food, cosmetic, and pharmaceutical industries ([14], pp. 37–70).

Solvents used as an extractant must be selective, readily available, low cost, and harmless.

Of the common methods for extracting extracts from plant materials with solvents, the following can be cited.

Maceration describes the soaking of solid plant material in a solvent at room temperature for a specified time. Thus, the solid is simply in contact with the solvent without any movement.

Usually, the extraction time is long and the efficiency is low. This can be explained by the fact that maceration is a process in which there is an equilibrium between the concentration of the extractable component in the plant material and the extraction

solvent. This means that the extraction occurs until the equilibrium concentration of the extractable components in the solvent is reached. However, depending on the properties of the solvent, a significant amount of the desired components may remain in the plant material. Complete recovery requires repeated addition of fresh solvent, resulting in high specific solvent consumption.

If maceration is carried out at a constant elevated temperature, the method is called assimilation (**Figure 1**) ([15], pp. 310–314; [16], pp. 517–554; [17], pp. 377–387; [18], pp. 8615–8627; [19], pp. 136–137).

Disadvantages of the method: duration of the process; insufficient depletion of raw materials, low yield of biologically active substances.

A further extraction technique is infusion. Here, the solid is immersed in a heated solvent, and the process proceeds without boiling, followed by cooling of the suspension. The most striking example of an infusion is the preparation of tea. In the case of a boiling solvent, the extraction method is called decoction. However, these methods are only applicable to thermostable compounds. However, it is a very fast and sometimes unavoidable extraction method ([14], pp. 37–70).

Unlike previously described techniques, percolation is a method of exhaustive coupling by extraction from plant material. For this, solid plant material is used, and the solvent passes through the fixed layer. As a rule, this process occurs under the action of gravity from top to bottom. In addition, adequate extraction efficiency can be achieved by recycling the extraction solvent and passing it through the plant multiple times. The advantage of this method is the relatively low mechanical stress on the hard material. Moreover, additional filtration is not required as the extract contains no particulate solids. The most striking example of percolation is the preparation of coffee in a coffee machine ([14], pp. 37–70; [15], pp. 310–314). Disadvantages of the method: energy consumption when supplying the extractant to the percolator (**Figure 2**).

The percolation method includes three consecutive stages: wetting of raw materials (swelling of raw materials), infusion, and percolation itself.

Another comprehensive method for extracting compounds using a suitable solvent is the Soxhlet method.

Typically, this method is performed in a laboratory setting to determine the total amounts of compounds that can be dissolved in the solvent used. The Soxhlet installation is shown in **Figure 3**.

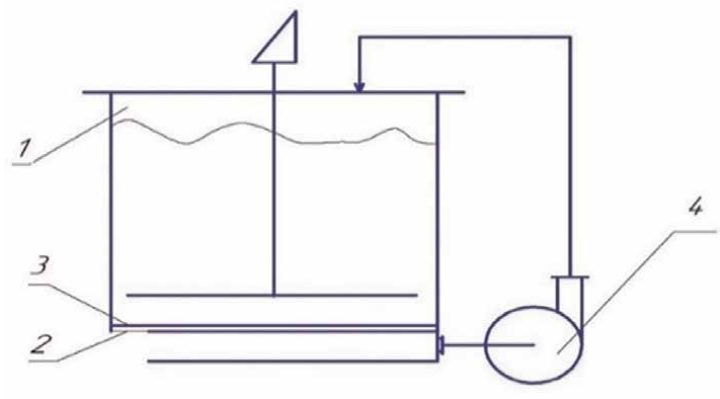

Figure 1.
Schematic diagram of maceration with extractant circulation. (1 - maceration tank; 2 - false perforated bottom; 3 - filter material; 4 – Pump).

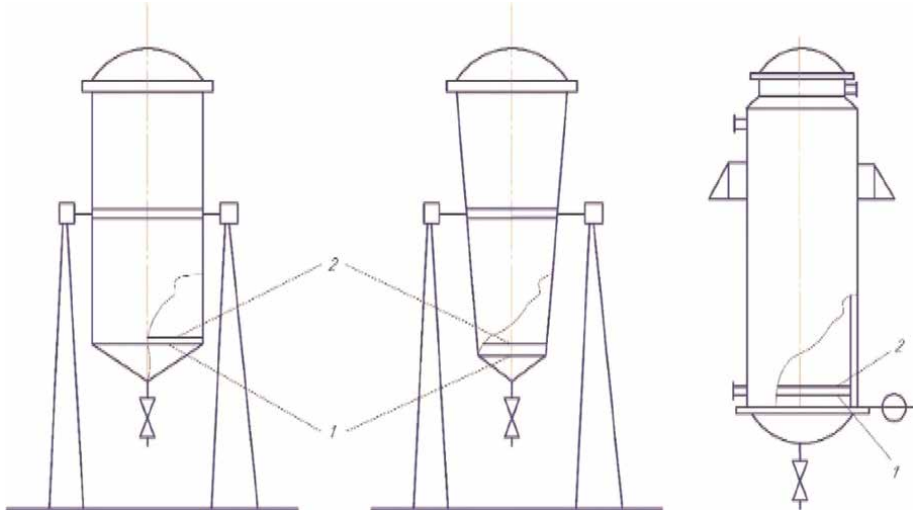

Figure 2.
Scheme of percolators-extractors. 1 - false bottom (perforated mesh); 2 - filtering material (burlap, linen, etc.).

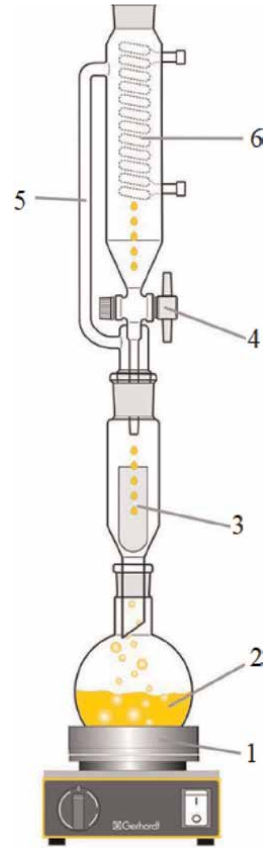

Figure 3.
Soxhlet laboratory setup for the extraction of plant material. 1 – Hotplate; 2 - solvent; –; 3 – Extraction thimble with sample; 4 – Sealing valve 5 - steam riser pipe– 6 – Condenser.

The previously extracted solid plant material is placed in a porous extraction thimble. It mainly consists of cellulose fibers. After that, the vessel is placed in the chamber of the Soxhlet extractor. The solvent in the lower flask is then heated to boiling. The steam vapor passes through the ascending pipe to the condenser. There, the pure solvent condenses, enters the extraction vessel, and comes into contact with the plant material. A certain amount of solvent, the chamber is emptied through a special siphon. Decomposition of components can also occur in the bottom flask, as the solvent is kept at its boiling point for several hours ([15], pp. 310–314; [16], pp. 517–554; [19], pp. 136–137; [20], pp. 169–198).

Disadvantages of the method: relative high cost of equipment, increased consumption of the extractant, high energy costs, the threat of atmospheric pollution with a volatile organic solvent.

There are various methods to speed up and improve the extraction methods described above. The use of microwaves and ultrasound as a stimulator-intensifier of the process [21].

When using ultrasound in the extraction process in the "solid-liquid" system, the main mechanism is the destruction of the surface structure of the material ([20], pp. 68–87; [22], pp. 10–23), diffusion intensification ([23], pp. 124–130), capillary sound effects, and exposure to acoustic microwaves ([24], pp. 231–255), their passage through the cells ([25, pp. 134–149) and local thermal effects ([22], pp. 10–23; [24], pp. 231–255; [26], pp. 656–659).

Disadvantages of the extraction method using ultrasound: high cost of equipment, increased energy costs, indifferent to active substances, target components.

In recent years, extraction with the help of microwaves has been intensively studied and successfully applied in the "solid-liquid" method of extraction of plant materials. Microwave radiation can be used to rapidly extract certain classes of plant compounds ([27], pp. 1–14). Microwaves directly affect the water in the plant matrix cells. These cells are destroyed by the high pressure generated by the microwaves, releasing the plant compound. The substance is dissolved in an organic solvent and then fed for processing ([14], pp. 37–70; [28], pp. 530–554; [29], pp. 708–716).

The extraction can also be carried out with a microwave-absorbing solvent. However, high heat should be avoided to prevent thermal decomposition of plant compounds ([15], pp. 310–314). Microwave extraction has a number of advantages over traditional methods. First of all, compounds can be extracted more selectively and faster. At the same time, the consumption of energy and organic solvents is reduced ([13], pp. 517–554; [14], pp. 37–70; [15], pp. 310–314; [30], pp. 105–113).

2. Features of technological parameters and factors of the extraction process

During the extraction of biologically active substances from plant materials with supercritical fluids, various individual compounds are involved, which raises the question of the effect of these or other components of the initial raw materials during the extraction process ([31], pp. 47–65; [32], pp. 101–105).

The general patterns of extraction by liquefied gases have been studied for raw materials with a variety of textures. To date, under different conditions, scientists have extracted more than a hundred different biologically active substances from plant materials, of various histological structures ([32], pp. 47–65; [33], pp. 101–105).

Factors affecting the extraction with liquefied gases are: temperature and pressure, viscosity of the solvent, grinding and moisture content of the material, infusion time, duration, and hydrodynamics of the process ([31], pp. 47–65; [33], pp. 55–62; [34], pp. 23–32). Modification of important parameters of the extraction process - provides an opportunity to obtain products of the desired composition ([35], pp. 259–262; [36], pp. 70–93; [37], pp. 50–55).

Influence of temperature and pressure. Supercritical extraction is based on diffusion processes, where the rate of the final mass transfer, in particular, is determined by the values of the diffusion coefficients at each stage, otherwise depends on their diffusion resistances. The total resistance to mass transfer ($R_{tot.}$) is the sum of internal resistance ($R_{int.}$), resistance of the diffusion layer (sub-layer) ($R_{dif.\ layer.}$), and convective resistance ($R_{conv.}$):

$$R_{tot.} = R_{int.} + R_{dif.layer.} + R_{conv} \quad (1)$$

Values $R_{dif.\ layer.}$ and $R_{conv.}$ (the second and third stages of extraction) are small and depend on the hydrodynamic conditions of the medium. On the efficiency of extraction at the first stage, the viscosity of the extractant, temperature, internal structure of the material, particle size of the extracted material and other factors are of decisive importance ([31], pp. 47–65).

Liquefied gases, having a low viscosity (1–2 orders of magnitude less than that of alcohol and water), excellent wetting and permeability, to a greater extent than other liquid extractants, affect the diffusion rate and solubility of the intracellular content contained in the material. This dependence is explained by the fact that with a decrease in viscosity, the diffusion coefficient (D) in the Einstein equation increases proportionally:

$$D = \frac{k \cdot T}{6 \cdot \pi \cdot \eta \cdot r} \quad (2)$$

whereat k – Boltzmann's constant, 13805, Dj/K; T – absolute temperature, °K; π – 3,14; η – fluid viscosity, Pa·s; r – diffusing particle radius, m.

Temperature and pressure during the extraction of liquid CO_2 in saturation, determine the chemical composition and quantitative yield.

Effect of temperature: Increasing the temperature at constant pressure leads to a decrease in the density of the liquefied CO_2, thus reducing the solubility of the solvent ([31], pp. 47–65).

With an increase in the pressure of the CO_2 extraction process, the extract yield increases.

The dependence of the increase in extraction efficiency on the increase in the temperature of the process is known, when the thermal movement of molecules is accelerated and the viscosity of the liquid components of the system decreases ([38], pp. 56–73).

Based on the position of thermodynamics that the critical point of a binary mixture is characterized by the vanishing of the first and second derivatives of the chemical potentials of the components, and from the fact that they are the driving force of isothermal molecular diffusion, the preferred development at this point is a significant slowdown in the extraction process.

Influence of the degree of grinding of raw materials. With flow extraction at a rate corresponding to the independent flow of the solvent through the plant material, the

yield of components, other things being equal, was increased by 1.5–3 times and reduced in time as a result of a decrease in the particle size of the raw material ([38], pp. 56–73; [39], pp. 80–84; [40], pp. 23–40).

The larger the contact surface of the phases, the faster the extraction. But very fine powders for extraction cannot be used:

1. A large amount of heavy substances, insoluble particles, and colloids pass from plant powders (destroyed cells) - a cloudy liquid that is difficult to clean is obtained;

2. The fine powder forms a thick mass with the solvent, which resists the passage of the solvent (**Table 1**).

Influence of extraction duration. All production processes are efficient if they are short-term. The concentration of the obtained components is influenced not only by the level of crushing of the material but also by the duration of the extraction. For each type of plant material, under all other identical conditions, this value is characteristic. The study of the kinetic regularities of the extraction process with liquefied gases gives an idea of the rate of extraction of biologically active substances from plant materials.

Analyzing the dependence of the change in the content of extractable substances in the raw material on the duration of extraction, two periods of the extraction process can be distinguished. The first is the period of fast extraction, during which the process proceeds at the highest speed, and the second is the period of slow extraction, the speed of which is much lower ([40], pp. 23–40).

The time of the second extraction period is 35–40 minutes, characterized by the extraction of biologically active substances contained in microcapillaries and in the interior of intact cells. During this time, 5–10% of the components remaining in the plant material are removed. Diffusion of substances from whole cells of the material takes a long time, because of this, the duration of extraction is set by the diffusion coefficient.

Influence of persistence. The quantitative yield of the sum of extractive substances or an individual substance is influenced not only by the degree of grinding of raw materials but also by the time of the process. For each type of raw material, with all other conditions being equal, this value is individual. The low viscosity of the extractant, a large number of destroyed cells (due to the fact that in most cases the degree of grinding of raw materials is 0.25–0.50 mm) leads to the fact that with an increase in the time of infusion, not pure extractant penetrates into the cells, but a concentrated thick solution of cellular contents ([31], pp. 47–65).

Influence of humidity of extracted raw materials. The concentration of biologically active substances during extraction with liquefied gases significantly affects the moisture content of the plant material. How low the moisture content of the material, the more the concentration of the output of biologically active substances. By crushing

grass, leaves, flowers	stems, roots and bark	fruits and seeds
3–5 mm	1–3 mm	0.3–0.5 mm

Table 1.
The degree of grinding in different plant materials.

extraction time, min	The yield of extractable substances (%) at personal movement speed (cm^3)	
	5	10
45	3.73	3.79

Table 2.
Yield of extractable substances at different speeds.

plant material on a roller crusher with a moisture content of more than 10%, the extraction of substances from this material is difficult.

Influence of the speed of movement of the solvent on the yield of extracted substances. The significance of the coefficient of numerical mass transfer of the components is determined by the hydrodynamic conditions of the extraction process. As high as the speed of movement of the solvent, so much more concentrations of the substance pass due to convection diffusion (**Table 2**).

An analysis of the effect of the ratio of raw materials: extractant showed that the amount of fresh solvent does not have a significant effect on the residual content of extractives in the raw materials, in contrast to, for example, the degree of grinding of the raw materials.

The density of the raw material layer in the extractor is also of great importance; with an increase, the transverse unevenness of the transition of the extractant to stagnant zones increases, where the solvent either does not move at all, or moves at a low speed, which slows down the extraction in such places.

Mixing significantly reduces the thickness of the boundary layer, increases the coefficient of external diffusion, and the volumetric velocity of the liquefied gas.

The limiting factor for the widespread introduction of the extraction of plant materials with liquefied and compressed gases is the underestimation of the biological value of the products obtained and the lack of specially designed standard equipment, control systems and safety of these processes ([31], pp. 47–65).

3. Analysis of the process and design of extraction plants using CO_2

The development of methods of energy-saving technologies that allow obtaining new high-quality products in the pharmaceutical, perfumery, cosmetology and food industries is due to the acute social need for high-quality medicines and food products, as well as in environmentally friendly industries ([41], pp. 16–27).

The determining factor in the production of high-grade food products is the enrichment of their composition with the missing natural ingredients.

Analysis of scientific, technical and patent literature shows, on the one hand, the prospects of using liquefied and compressed inert gases as extractants of valuable components from vitamin-containing plant materials, on the other hand, the absence of a unified system of scientifically based use in the food industry – carbon dioxide.

In addition to technological advantages, the use of carbon dioxide as a solvent is economically beneficial, since it is a relatively cheap and readily available volatile solvent.

Despite significant advances in the field of subcritical CO_2 extraction, there is today a large gap between research and development and the speed of their implementation in industry. A number of studies have been carried out on the extraction of

biologically active components from raw materials with carbon dioxide in a pre- and supercritical state ([42], pp. 251–253; [43], pp. 26–29; [44], pp. 108–114; [45], pp. 136–148; [46], p. 324; [47], p. 207). Within the framework of the second industrialization of industry, the prospects for the extraction of target substances from raw materials with gases in various phase states are determined ([48]; pp. 368–371; [49], pp. 36–41; [50]; [51], p. 106, pp. 196–199; [52], pp. 35). The analysis of the combinatorial algorithm of the CO_2-extraction process was carried out ([53], pp. 100–102; [54], pp. 124–125; [55], pp. 217–223). Much attention is paid to the technical equipment of extraction gas-liquid enterprises ([56], pp. 1354–1365; [57], p. 445). Over the past decade, several examples of pilot large-scale projects have been reported to implement a technological and economic strategy for the production and use of CO_2 extracts ([54], pp. 124–125; [58], pp. 260–269; [59], pp. 74–77; [60], pp. 8–33; [61], p. 43).

In general, pure chemicals can be in various states of aggregation depending on pressure and temperature. On **Figure 4** shows the phase diagram of carbon dioxide (CO_2). Using this diagram, you can understand what state a substance is in at a given pressure and temperature. In particular, there are four different regions in which the substance is in one phase: solid (t), liquid (l), gaseous (g), and supercritical (SC) ([62]; pp. 119–134).

The point at which the evaporation curve ends is called the "critical point." It is characterized by a critical temperature Tc and a critical pressure Pc ([62], p. 119–134; [63], pp. 1766–1778). The critical point of carbon dioxide is at T_c = 31,1°C и P_c = 7,38 MPa ([64], [65], pp. 15–16).

For the development of extraction technology, PVT data (specific volume depending on pressure and temperature), viscosity, diffusion coefficients, solubility, density dependence on V and T are of great importance.

At 40°C, the gas density changes sharply within the limits of up to 20 MPa, it increases to 785.13 kg/m^3, i.e. exceeds the density of liquid carbon dioxide (at 20°C and P = 5.9 MPa). At a pressure above 30 MPa, the gas density increases more smoothly (at 40 MPa 924.05, kg/m^3, at 60 MPa 1000 kg/m^3) ([66], pp. 8–12). The density of a gas depends not only on pressure but also on temperature. At a pressure of 20 MPa and a temperature of 40°C, the gas density is ρ = 785.13 kg/m^3, at 100°C - ρ = 481.3 kg/m^3, at 200°C - ρ = 258.15 kg/m^3 (**Figure 4**).

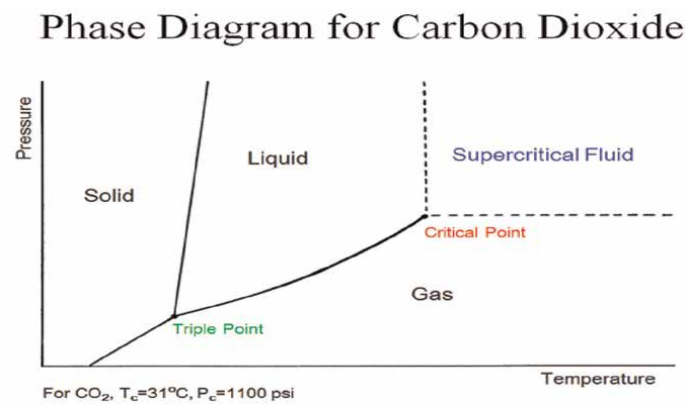

Figure 4.
Phase diagram of carbon dioxide.

Supercritical extraction technology finds its application in food, cosmetics and pharmaceutical industries ([64], [67], pp. 977–982; [68, 69], pp. 1–106.).

Extraction with supercritical carbon dioxide is used to extract volatile (e.g. essential oil) and/or non-polar (e.g. fats, waxes) compounds from plant materials. There are many variables that must be considered in order to optimize the extraction yield and selectivity of supercritical extraction ([29], pp. 708–716; [70], pp. 771–782; [71], pp. 2–24; [72], pp. 1–37). However, in most cases of extraction from plant materials, the diffusion of the target compound from the matrix is usually the rate-limiting step ([73], pp. 320–325). This can be avoided either by increasing the temperature and hence the volatility of the compound, or by using high solvent densities. To achieve good selectivity of SC extraction, the density of the solvent must be carefully controlled ([16], pp. 517–554; [70], pp. 771–782; [74], [75], pp. 1–15, [76], pp. 143–149, [77], pp. 1–5; [78], pp. 58–69; [79], pp. 27–51).

Modifiers are used to improve the extraction ability of supercritical – CO_2, among which methanol is the most commonly used co-solvent for supercritical extraction due to its polar properties and good miscibility with CO_2 ([70], pp. 771–782). Longer extraction times increase the extraction yield of most compounds. A distinction should be made between static and dynamic extraction. It has been shown that a preliminary static extraction step can increase the yield as the contact of the sample with the solvent is improved ([71], pp. 2–24; [76], pp. 143–149). The flow rate of supercritical carbon dioxide through the extraction vessel also has a strong influence on the extraction efficiency ([71], pp. 2–24; [72], p. 1–37). Reducing the particle size of the plant material leads to an increase in surface area and facilitates the process. However, excessive grinding of the solid can make extraction difficult due to re-adsorption of the target compounds on the surface of the matrix. ([71], pp. 2–24; [76], pp. 143–149; [80], pp. 22–26).

Our country is very rich in plant raw materials for the production of food biologically active substances, pharmaceutical substrates, as well as perfumery raw materials. But so far no thorough research has been conducted on this topic in Uzbekistan.

Improving the technique and technology of extraction of plant raw materials with carbon dioxide is possible on the basis of research on both the extraction process itself, focused on local raw materials, and the operation of the entire complex of apparatuses of the extraction plant is very important in terms of obtaining high-quality products at a relatively low cost ([81], pp. 87–95).

The criteria for choosing a new extraction technology with liquefied CO_2 are: separation by traditional methods is impossible or expensive or unsatisfactory; – at least one of the properties of liquefied and compressed gases solves the problem, the value of the resulting target product justifies the economic costs.

Liquid CO_2 does not support the vital activity of microorganisms and molds, which makes it possible to obtain sterile products even when using raw materials contaminated with microorganisms, liquid CO_2 is thermally stable at normal temperatures, and is chemically inert. The separation of the solvent from the extract is possible either by lowering the pressure or by heating, which converts liquid CO_2 into a gaseous state, while CO_2 is released - extracts ([80], pp. 22–26).

The use of liquefied carbon dioxide as an environmentally friendly solvent is one of the rapidly developing areas in the development of the latest state-of-the-art gas-liquid technologies.

In the supercritical state, gases are substances used as solvents at temperatures and pressures exceeding the critical value. Many gases have a critical temperature close to

room temperature and a critical pressure in the range of 5.0–8.0 MPa, which makes them very convenient and inexpensive to use in industry.

The extensive formation of technologies using supercritical gases is associated with the use of their unique properties. This is a combination of the properties of gases at high pressures (low viscosity, high diffusion coefficient) and liquids (high dissolving power). With this, the dissolving power of supercritical gases is very sensitive to changes in pressure or temperature ([82], pp. 47–49).

Very prominent is the possibility of carrying out a rapid mass transfer, carried out due to the low viscosity and high diffusion coefficient; the combination of low interfacial intergrowth with low viscosity and high diffusion coefficient, which makes it easier for supercritical gases to penetrate porous media compared to liquid solvents. The main condition is the easy decomposition of liquefied gases and substances extracted in them by pressure reduction.

Depending on the method of phase contact, extractors can be divided into three groups: stepped or sectional, differential-contact, and mixing-settlement ([5], p. 256; [83], p. 388).

- In international technological practice, installations for carrying out CO_2 - extraction are known, which can be cited as an example:
- for extraction with liquefied gases at doktric parameters of pressure and temperature;
- for extraction with liquid gases at a pressure above and a temperature below the critical one;
- for extraction with compressed supercritical gases;
- with mixed processes – multi-stage ([60], pp. 8–33; [84], pp. 21–26; [85], pp. 394–399; [86], pp. 166; [87], pp. 388–389).

At present, CO_2 extraction plants have a number of disadvantages. For example, in the extraction shop of the Yuventa company (Krasnodar), extraction modules manufactured in the last century were installed. Part of the equipment of the modules is not made of stainless steel but of ferrous metal. There is no installation for mixing raw materials with solvents ([46], p. 324; [88], pp. 43–46).

The most advanced equipment is installed in the extraction shop of Caravan LLC (Krasnodar). It provides for the delivery of liquid carbon dioxide in tank trucks with a capacity of up to 8 tons, high-pressure extractors with a capacity of up to 150 liters, and the possibility of quickly removing CO_2 vapor from the evaporator to the condenser. The disadvantage of the workshop equipment is an irrational system for loading and unloading raw materials into extractors ([89], p. 152; [90], pp. 94–97).

The universal extraction module of Caravan Company LLC [91] is designed for the extraction of biologically active substances from plant raw materials with liquid CO_2.

In the experimental plant of the Research Institute “Mir-Prod-Mash” (Moscow), extracting modules were produced, which include 2 extractors of 10 liters each, an evaporator and a condenser. However, the design of the module does not provide for intensive mass transfer in the “solid-liquid” system; as a result, the extraction of components from the raw material lasts 8 hours.

The formation of effective equipment samples is associated with the primary development of extraction modes and designs of individual units at laboratory facilities.

A schematic diagram of the installation for the extraction of ingredients from tea and medicinal raw materials with liquid CO_2 is shown in **Figure 5**.

A feature of the improved laboratory setup is the inclusion of a vacuum pump 13 in the circuit, which allows air to be evacuated from the extractor and plant material weighed (**Figure 6**). Placement of miscello-receivers outside the body of the sealed apparatus makes it possible to select fractions of extractive substances depending on the duration of the process of extracting plant materials.

Distinctive features include lining the internal surfaces of equipment with ceramic, metal-ceramic and carbon-plastic coatings, improving the drainage system of extractors due to drainage ceramic tubes, increasing the heat exchange surface of the evaporator and condenser ([89], p. 152; [90], pp. 94–97).

Figure 6 shows the hardware-technological scheme for the production of CO_2 extracts, created in the conditions of the extraction shop of Caravan Company LLC (**Figure** 7).

The developed devices ensure the achievement of results in the field of CO_2 - the extraction of significant ingredients from plant material.

The patterns of interaction, functioning and development of technological processes based on the interaction of food products with CO_2 in stable or changing phase states have been established, which made it possible to develop the scientific foundations of engineering solutions in the field of engineering and technology of CO_2 - processing of plant material [92].

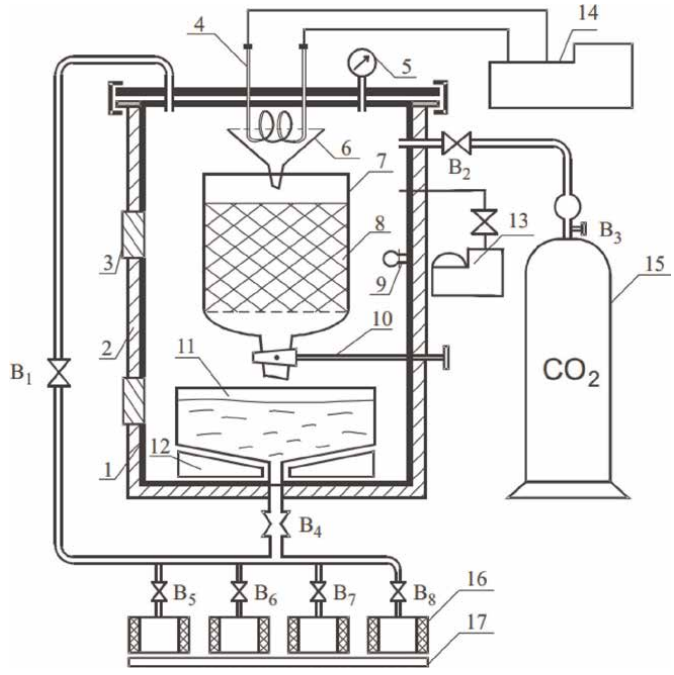

Figure 5.
Schematic diagram of the installation for extraction with liquid carbon dioxide.

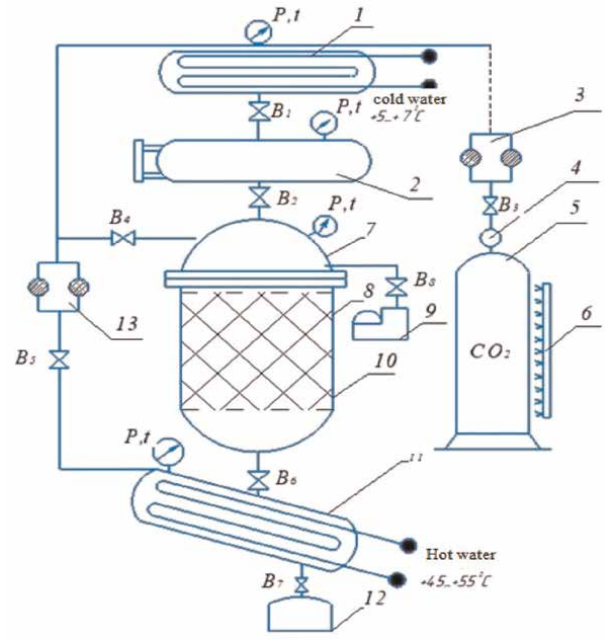

Figure 6.
Improved batch extraction plant.

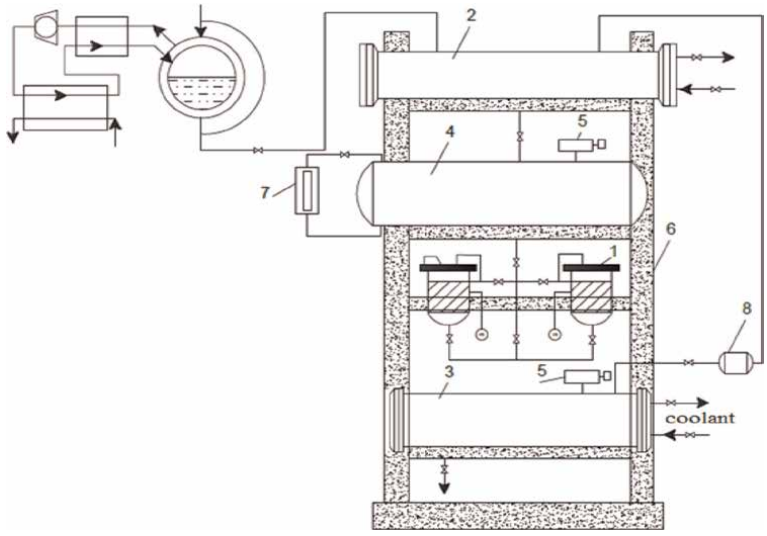

Figure 7.
Hardware-technological scheme for the production of CO_2 extracts.

Apparatus and equipment for carbon dioxide extraction are classified in the following works ([79], pp. 27–51; [92], [93], p. 495; [94], [95], p. 390).

The following scientific study describes a multi-stage (subcritical / supercritical) pilot plant (**Figure 8**) from Separeco S.r.l. (Italy) ([96], pp. 358–363).

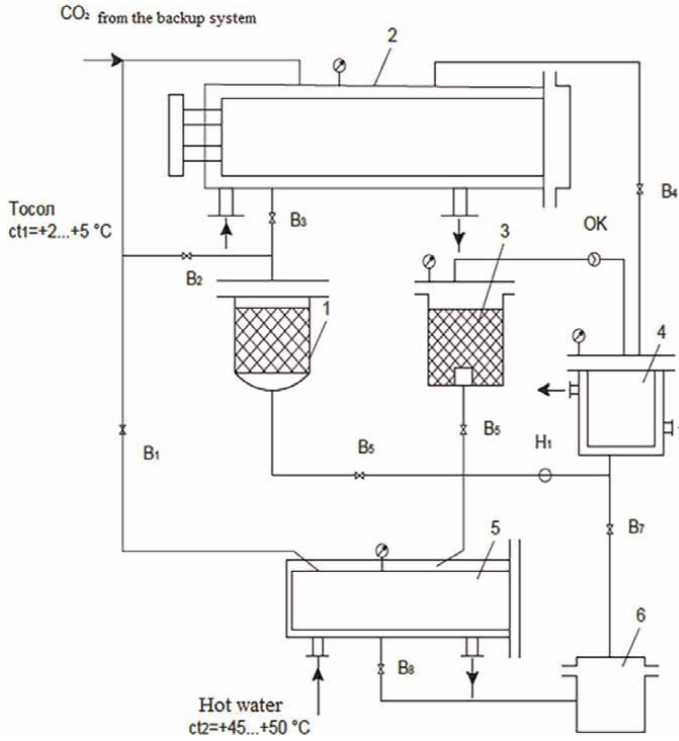

Figure 8.
Combined scheme of sub- and supercritical extraction of valuable components from plant materials.

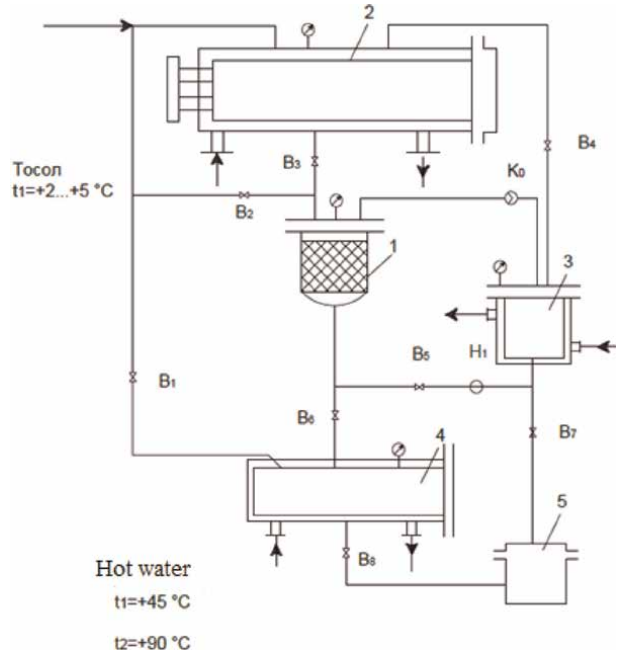

Figure 9.
Scheme of the modernized installation for studying the process of CO_2 extraction in the pre- and supercritical state of carbon dioxide.

According to the principle of operation, the devices can be divided into laboratory, experimental (desk or pilot) and industrial ([97], pp. 6–42; [98], p. 240).

The scientific work [98] describes in detail the process of assembling an apparatus for supercritical fluid extraction, created and manufactured at the Technical University Hamburg Harburg (TUHH) ([99], pp. 210–247).

In the construction of the following work, a greater porosity and porosity of plant raw materials is achieved, which contributes to a higher product yield and intensifies the process, reducing the extraction time [90, 91].

And the following study shows the installation (**Figure 9**) equipped with a device for superheating the solvent and a high-pressure pump, which makes it possible to extract the ingredients sequentially in the sub- and supercritical states of the solvent ([100], pp. 13–21).

Extracts obtained from plant materials using carbon dioxide are used in the food, perfume and pharmaceutical industries, as they are more environmentally friendly than extracts extracted by other traditional methods.

4. Licorice root and its chemical composition

Currently, reforms are being carried out in Uzbekistan in the field of protection of medicinal plants, rational use of natural resources, creation of plantations for growing medicinal plants and their processing [101].

In Uzbekistan, 4.3 thousand plants, of which 750 varieties are medicinal, 112 varieties are registered for use in scientific medicine, 70 species are used in the pharmaceutical industry.

By presidential decree No. 4670, reproductive organs, that is, seedlings, typical gray soils suitable for flat lands, moisture-loving, saline (cryptohalophytic) plant - licorice or licorice root (Glycyrrhiza glabra L.) are recommended for planting in all regions of the Republic of Karakalpakstan, Syrdarya, Khorezm areas [101].

There are about 13 types of licorice root (licorice) in the world. The most common species are: licorice naked (Glycyrrhiza glabra), Ural licorice (Glycyrrhizauralensis Fisch) and Korzhinsky licorice (Glycyrrhizae KorshinskyiGrig). Licorice is the most popular among them, its roots contain the largest amount of biologically active substances (BAS) ([102], pp. 11–13; [103], p. 314).

Licorice root is a rather high-calorie product, 100 g contains 375 kcal. It contains no proteins, almost no fat (0.05 g), a small amount of fiber (0.2 g) and a lot of carbohydrates (94 g). Dried licorice roots have been widely used since ancient times as a food flavoring agent, sweetener, and for various medicinal purposes [104, 105].

The art of healing with the help of licorice root extracts, developed by Eastern medieval medicine, is summarized by Avicenna. Avicenna considered the nature of the licorice root to be balanced ([106], pp. 150–155).

Licorice root, as well as biologically active substances isolated from them, has a wide range of pharmacological properties ([107], pp. 13–26). To date, the results of more than 2 thousand scientific studies on the licorice root and its biologically active substances have been published, which confirms the great interest of pharmacologists in plants of this genus in many countries of the world. To date, licorice root in all countries is widely used in the food, pharmacological, cosmetology industries ([108], p. 210; [109], pp. 189–191; [110], pp. 108–114; [111], pp. 21–27).

Not to mention the fact that interest in licorice root has grown incredibly sharply over the past 20 years and it is in first place among herbal preparations ([112], p. 378).

Pharmacological and chemical properties of licorice root have been comprehensively studied by I. A. Muravyov, K. Z. Zakirov, V. I. Litvinenko ([113], p. 191).

In ([102]; With. 11–13) for the first time studied the chemical composition of the roots of licorice growing in the Samara region. Glycyrrhizic acid (triterpene saponin) and flavonoids - licurazid, liquiritin, liquiritigenin (flavonones) were isolated and identified from the roots of the licorice root using UV, 1H-NMR spectroscopy, and mass spectrometry, the results of chemical transformations; isoliquiritin, isoliquiritigenin (chalcones); ononin, formononetin (isoflavones).

A processed product, that is, licorice root extract, is very popular. Experts are unanimous that the global demand for finished products made from licorice root will constantly increase ([114], p. 466).

The scientific literature describes about 80 triterpenoids and over 300 individual phenolic compounds, several dozen polysaccharides, amino acids and many other substances that have a variety of pharmacotherapeutic properties found in the licorice root ([107], pp. 13–26; [115], pp. 16–19; [116], p. 83; [117], pp. 152–158; [118], pp. 1954–1969).

The main active ingredients and biologically active components of licorice root are triterpene glycosides (the most important of them is glycyrrhizic acid), the content of which can reach 25% by weight of dry material, various phenolic compounds, which account for 3–5%, glabridin and carbohydrates. The total content of extractive substances can reach 40% ([119], pp. 55–59).

The quality of the licorice root is standardized by the State Pharmacopeia (GF): the content of extractable components extracted by 0.25% ammonium hydroxide solution must be at least 25%, moisture not more than 14%, ash not more than 8%, glycyrrhizic acid – not less than 6% ([107], pp. 13–26; [120], pp. 11–13).

The licorice root contains biologically active substances such as: flavonoids up to 5.0%, carbohydrates up to 34.0%, proteins - up to 10.1%, amino acids up to 12.71%, including asparagine up to 4.0%, lipids up to 4.7%, vitamin C up to 3.1% ([107], pp. 13–26; [121], p. 175).

Glycyrrhizic acid with high-intensity sweetness (about 50 times more than sucrose), widely used in all countries as a biological additive in the food, beverage, cosmetic, pharmaceutical and tobacco industries ([122], p. 271; [123], pp. 55–72). The content of glycyrrhizic acid in licorice roots varies in the range of 2–24% ([124], pp. 166–168). Glycyrrhizic acid is highly soluble in ethanol and hot water, insoluble in cold ([120], pp. 145–158; [125], pp. 87–94; [126], pp. 32–34). Melting point (Tm) of glycyrrhizic acid is 220° C). The UV spectrum of glycyrrhizic acid shows that its maximum absorption peak is in the region of 254 nm ([127], p. 1331; [128], pp. 100–104; [129], pp. 87–91).

Glycyrrhizic acid was first extracted from the licorice root by the French scientist Pierre Jean Robiquet in 1809 and gave the name glycyrrhizin. After Z. Russin named glycyrrhizic acid in 1876 (**Figure 10**) ([107], pp. 13–26).

Glycyrrhizic acid is active against a wide range of viruses, including herpes, corona, alpha, and flaviviruses, human immunodeficiency virus, type I poliovirus, vesicular stomatitis virus, and influenza A virus ([128], pp. 100–104; [130], pp. 1256–1259; [131], p. 333; [132], pp. 199–206).

Nowadays, the following phenolic compounds have been isolated and established from the licorice root: simple phenols (phenol, resorcinol, pyrogallol), phenolcarboxylic acids, hydroxycinnamic acids (ferulic acid, cinnamic acid, synapic acid).

To date, over 50 different flavonoids have been extracted and identified from licorice root. ([131], p. 333; [133], pp. 6–8; [134], pp. 34–80; [135], pp. 1027–1030).

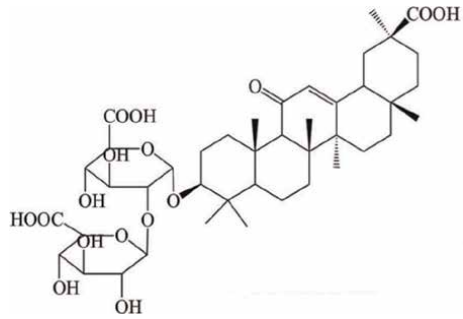

Figure 10.
Structural formula of glycyrrhizic acid.

The content of flavonoids in licorice roots is 3–6%. The work 136 presents the main flavonoids of liquorice root and their characteristics ([134], pp. 34–80).

Carbohydrates extracted from licorice root: established ([124], pp. 166–171; [135], pp. 1027–1030) that the carbohydrate content in the licorice root reaches up to 40%.

In ([136], pp. 10–24), the author studied the properties and chemical composition of various morphological organs of liquorice collected from the northern regions of the Republic of Uzbekistan. The chemical composition of licorice root is presented in **Table 3**.

Traditionally, licorice root is most actively (due to climatic features) grown in the north-west of Uzbekistan - in Karakalpakstan and the Khorezm region [137].

The president of the Japanese company, Naomasa Yoshida, emphasizes that the laboratories of Cokey Systems Co. Ltd. regularly test samples of licorice root from many countries, while licorice root from Uzbekistan is recognized as the best in the world in terms of its composition, thickness, smell and color and other quality attributes [138].

In Ref. ([139], pp. 1338–1340), the chemical characteristics of licorice root collected from Uzbekistan are given. A study of Glycyrrhiza glabra L. was carried out in Uzbekistan in August 2001 and above and below ground parts of the plant were collected from a site near Yangiyer, 100 km south of Tashkent. Licorice, which grows in Uzbekistan, contains glycyrrhizin up to 6.1% of dry weight.

In the study ([140], pp. 677–680), the author also identified flavonoids glycionide A and B - glucose (up to 15.2%), sucrose (up to 11%), starch, resinous substances,

Name of substances	Content, %
Substances extractable with a mixture of alcohol / benzene	13.45
Easily hydrolysable substances	11.20
Substances that are difficult to hydrolyze	57.60
Reducing sugars	15.50
Cold water extractable	44.90
hot water extractable	49.40
Cellulose according to Kirchner	29.60
Nitrogen	0.97

Table 3.
Chemical composition of licorice root.

Name of substances	Content, %
Extractives	22.8–44.1
Triterpenoids	7.3–23.6
Carbohydrates (glucose, sucrose, starch)	18.2–34.0
Flavonoids	3.0–4.0
Steroids	1.5–2.0
Ascorbic acid	1.1–3.1
Essential oils	1.5–2.0
Asparagine	1.0–4.0
Resinous substances	1.7–4.1
Fats and fat-like substances	0.2–4.7
Protein	6.2–10.1
Comedy	1.5–6.5
Bitterness insoluble (in water)	1.8–4.0
Ash (total)	4.9–9.7

Table 4.
The main biologically active substances of licorice root.

gums. There are many organic acids in the licorice root – salicylic, synapic, ferulic, caffeic and others. Coumarins, alkaloids, tannins, steroids, estradiol, vitamins C, B were also determined. K, Ca, Fe, Si, Sn salts are concentrated in the licorice root.

According to the literature, licorice root contains a number of biologically active substances, such as triterpene saponins, flavonoids, coumarins and other phenols, in accordance with **Table 4** ([141], pp. 30–51; [142], pp. 1868–1886). The total volume of extractive biologically active substances isolated from licorice roots reaches 40% of the mass of the feedstock ([143], pp. 55–59).

Most medicinal products based on licorice root require the introduction of modern methods of quality control into the regulatory documentation for the standardization of pharmacological substances of plant materials "Licorice Roots" ([107], pp. 23–31).

Development phase	Biologically active substances		
	glycyrrhizin acid	sugar	flavonoids
regrowth	7.67 ± 1.30	7.80 ± 2.12	1.15 ± 0.50
	8.49 ± 1.35	4.92 ± 1.50	3.60 ± 0.22
bloom	10.82 ± 1.81	9.19 ± 1.05	3.41 ± 0.85
	6.85 ± 1.60	5.64 ± 1.00	0.92 ± 1.00
fruiting	14.76 ± 0.50	6.65 ± 1.40	2.62 ± 0.46
	10.96 ± 2.00	8.59 ± 0.35	1.05 ± 0.35

Table 5.
The content of glycyrrhizic acid, sugars, flavonoids in licorice roots depending on the vegetation phase, % of absolutely dry weight.

regrowth	N	P	K	Ca	Mg	Cu	Mn	Zn
		%		g/kg			mg/kg	
bloom	3.38	0.23	0.32	12.37	5.22	9.63	23.50	20.63
	2.67	0.23	0.39	12.64	4.54	6.13	11.50	14.50
fruiting	3.08	0.32	0.35	13.62	1.91	5.56	14.75	20.50
	2.88	0.38	0.39	14.02	0.84	4.69	9.63	11.25

Table 6.
The content of mineral elements in licorice root, in terms of absolute dry weight (averaged data).

A solid extract is obtained by further evaporation of the thick extract to a solid state ([102], pp. 11–13; [144], [145], pp. 35–41). **Tables 5** and **6** show the characteristics of licorice root.

In the licorice root, an important structure is glycyrrhetinic acid, which is found in a natural compound – glycyrrhizin.

The main pentacyclic triterpenoid isolated from licorice root extracts, glycyrrhetic (glycyrrhetic) acid, is active against test microbes of staphylococcal, intestinal, and spore-forming groups ([146], p. 311). Glycyrrhetic acid has shown a protective effect against *C. albicans* fungal infection in mouse models ([147], pp. 310–315), as well as staphylococcal pneumonia caused by bacteria *Staphylococcus aureus* (**Figure 11**) ([148]; pp. 201–206; [149], pp. 241–248).

The antibacterial activity of sulfonamide GLA derivatives against gram-positive (*S. aureus*, Bacillus anthracis, Corynebacterium bovis) and gram-negative (*Klebsiella pheumonie*, *Proteus vulgaris*, *Escherichia coli*) bacteria has been established ([146], p. 311). However, the use of glycyrrhetic acid as a basic structure (scaffold) for the synthesis of its biologically active derivatives, as well as the study of the structure–activity dependence, is still a poorly studied direction ([148], pp. 201–206).

Phenolic compounds of licorice root have been sufficiently studied and widely presented in domestic and foreign publications ([147], pp. 1–158; [150], pp. 705–709; [151], pp. 4267–4272). Interest in licorice flavonoids is associated with their biological properties used in the production of medicines, food, technical and other products [152]. Interest in licorice flavonoids has especially increased over the past 20 years ([107], pp. 13–26; [116], p. 83; [153], pp. 7408–7414).

Essential oil (0,03%) ([154], pp. 5–14), it contains aldehydes, ketones, alcohols and their derivatives: ([155], pp. 1238–1241; [156], pp. 1179–1182); organic acids and

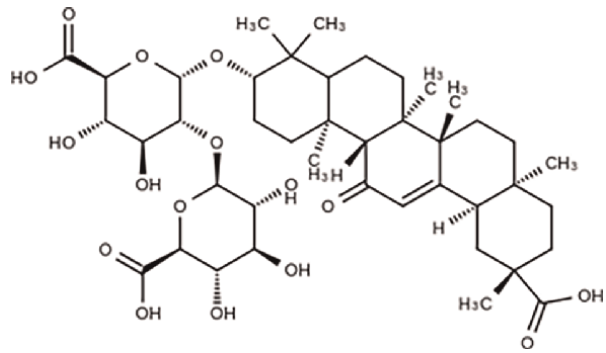

Figure 11.
Structural formula of glycyrrhetic acid.

macro elements, mkg/g					micro elements, mkg/g										
K	**Ca**	**Mn**	**Fe**	**Mg**	**Cu**	**Zn**	**Cr**	**Al**	**Ba**	**V**	**Se**	**Ni**	**Sr**	**Pb**	**B**
14.5	11.5	2.4	0.7	0.15	0.3	0.33	0.07	0.5	0.4	0.3	12.1	0.6	1.01	0.03	54.8

Table 7.
Macro- and microelements of licorice root.

their derivatives: propionic, phenylpropionic, caproic, caprylic, pelargonic acids and other compounds ([157], pp. 1179–1182); aromatic compounds ([154], pp. 5–14), tetramethylpyrazine ([156], pp. 1179–1182); higher aliphatic hydrocarbons: tetradecane; esters of higher fatty acids: ethyl talmitate, ethyl linoleate, ethyl linolenoate ([155], pp. 1238–1241); phenolcarboxylic acids and their derivatives: ferulic, synapic ([153]; p. 193), salicylic, salicylic acid acetate ([158], pp. 259–269); higher aliphatic hydrocarbons and alcohols (in the hydrolyzate): nonacosan, tetracosanol, octacosanol ([159], pp. 620–621); higher fatty acids (in the hydrolyzate): palmitic, oleic, etc. ([107], pp. 13–26; [160], pp. 159–162).

Licorice roots contain ash – 7.88%. **Table 7** shows the macro and micro elements of the root.

Licorice root is widely used in the food industry - extracts, syrups, as a sugar substitute and foam-forming drug in soft drinks (licorice extract is one of the main parts of Coca-Cola and Pepsi-Cola), beer, kvass, many drinks, are used in food preparation such as: coffee, cocoa, marinades, compotes, kissels, flour and whipped products, sweets, halvahs. They are added as a flavor additive - in the production of fish and as a bioadditive to long leaf and green tea. In Kyrgyzstan, they replace tea. In Japan – as a dietary antioxidant supplement; in Japan and Egypt – among component additives with bactericidal and fungicidal properties for food and drinks [157].

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Chapter 2

Natural Phenolic Compounds: A Review of Extraction and Analysis

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Abstract

Phenolic compounds are one of the major classes of secondary metabolites found in plants with enormous diversity of chemical structures and biological activities. They are classified into four main classes: phenolic acids, flavonoids, stilbenes, and lignans, and these compounds are unequally distributed in plant species with varied stability. Therefore, it is obviously necessary to select a proper extraction and analysis method to guarantee a high-yield extraction process and accurate analysis results. In recent years, more and more scientists have paid attention to either conventional or innovative methods for recovering phenolic compounds from the sample matrix. In order to give an overview of recent studies about phenolic compounds, this review will mainly focus on extraction and analysis methods and discuss some research directions that will receive more attention in the near future.

Keywords: phenolic, flavonoids, extraction methods, liquid chromatography, mass spectrometry

1. Introduction

Phenolic compounds are important secondary metabolites of plants and have various beneficial effects on human health [1]. Generally, these compounds are characterized by having at least one aromatic ring, which bears one or more hydroxyl groups. They could be classified into four major groups including phenolic acids, flavonoids, stilbenes, and lignans based on the function of the number of phenol units present, their carbon skeleton, and other structural elements binding these rings [2].

Phenolic acids are mainly divided into two main classes including benzoic acid derivatives (*p*-hydroxybenzoic, protocatechuic, vanillic, syringic acids, etc.) and cinnamic acid derivatives (chlorogenic, ferulic, caffeic, *p*-coumaric, sinapic acids, etc.). These compounds are found in various medicinal plants, fruits, and vegetables and are commonly present in bound form (amides, glycosides, or esters) and barely in free form [3]. For instance, caffeic acid—one of the most common cinnamic acid derivatives—is usually found in vegetables and fruits in bound forms with quinic acid, while ferulic acid is mostly found in cereals. In addition, phenolic acids are

considered strong natural antioxidant compounds, play a key role in protecting the organism against stress factors, and contribute approximately one-third of the phenolic compounds in human diets [4].

Besides, flavonoids are the most abundant group of polyphenols in nature with more than 9000 known structures [5]. The flavonoid skeleton includes two aromatic rings linked to a heterocyclic pyran or pyrone. In nature, they can be found in their free form, namely aglycones, polymerized or glycosylated forms. In addition, flavonoid compounds are classified into seven subclasses including flavones, flavanones, isoflavones, flavonols, chalcones, flavanols, and anthocyanins based on the degree of oxidation of carbon ring and the unsaturation of the heterocycle [6]. Furthermore, the hydrogens in the flavonoid skeleton could be replaced by many groups like hydroxyl, glycosyl, methyl, and acylated glycoside resulting in enormous structural diversity [7]. They exhibit a wide range of biological and pharmacological properties such as anti-inflammatory, anti-oxidative, anticarcinogenic, antimutagenic, antibacterial, antifungal, antiviral, and cardiovascular actions [6].

On the other hand, lignans are classified into two classes: lignans or neolignans, depending on the presence or absence of the 8,8′-bond between phenylpropanoid monomers [8]. They mostly exist as dimers in free form, while a few exist in glycoside form and could be found in rye, wheat, citrus fruits, etc. In recent years, because of their intriguing structures and biological activities like antitumor and anti-inflammatory characteristics, these compounds have attracted much attention from chemists and pharmacologists [4]. The last group of phenolic compounds is the stilbenes. They are characterized by a 1,2-diphenylethylene backbone and exist in small amounts in the human diet, mainly in the form of resveratrol. These compounds play a key role in some biological activities like anticarcinogenic, anti-inflammatory, and cardioprotective characteristics and could be found in trees, fruits (grapes and berries), peanuts, and red wine [1].

Given the wide variety of pharmaceutical and industrial applications (food processing and cosmetics industries) of phenolic compounds, the extraction and determination of these naturally gifted molecules in plants are essential to evaluate their health effects as well as contribute to food-quality management, food-product development, and nutritional and health monitoring [2, 9]. However, the extraction and purification of phenolic acids in plant matrices have many challenges because their solubility, stability, and separation properties are profoundly affected by their structural differences. Moreover, they could be found in free form or bound forms with various other plant matrix components [4]. In addition, the recovery of phenolics during the extraction process could also be reduced by the high level of enzyme activity in most plant matrices [1]. Therefore, the utilization of different extraction methods and appropriate extraction solvents are apparently significant in recovering these compounds. On the other hand, nowadays, phenolic compounds are commonly detected and quantified by using chromatography coupled with spectroscopy. In terms of separation, high-performance liquid chromatography (HPLC) and gas chromatography (GC) are the most used techniques due to their qualified reproducibility and accuracy [10]. Besides, ultraviolet/visible detector (UV/Vis), fluorescence detector (FLD), mass spectroscopy (MS), and nuclear magnetic resonance (NMR) spectroscopy are usually coupled with LC or GC systems for both quantification and identification purposes [2].

In this chapter, we briefly present the most common extraction techniques for the recovery of phenolics from plants (both conventional and unconventional methods), as well as provide updated information on different separation and

identification—quantification—methods regarding phenolic compound determination. Moreover, the most recent applications and research trends on this topic are also discussed, mainly corresponding to research papers published in the last 5 years (2019–2024).

2. Extraction methods

2.1 Conventional extraction methods

2.1.1 Maceration

Maceration is a conventional extraction method based on solid-liquid extraction. This method has been widely applied for decades to extract phenolic compounds from plant tissues by using a specific solvent with or without shaking or agitation to facilitate mass transfer, at room temperature or gentle heating for a period of time. The extraction process is solely carried out by molecular diffusion, and it's crucial to have enough extraction time, which can range from several hours to days, for the solvent to diffuse into the cell walls and dissolve phenolic compounds present in plant materials. Prolonged extraction time may cause the degradation of extracted compounds [11, 12]. In maceration, an appropriate extraction solvent is often ethanol, methanol, or water. If the extraction phase is water and the maceration period is long (more than 1 day), a small amount of an organic solvent (ethanol or methanol) should be added to the extraction solvent to prevent the growth of microorganisms (**Table 1**) [19]. In some cases, the combination of ethanol or methanol and water as an extraction phase could lead to a higher extraction yield of phenolic compounds instead of using a single solvent. For example, the extraction of phenolic compounds from *Momordica charantia L*. leaves utilizes an optimized condition with 70% methanol as the extraction solvent [20]. In another example, the optimal maceration parameters for determining the total phenolic content (TPC) in *Catharanthus roseus* L. were 30mg of dried *C. roseus* leaves in 0.72 mL of extraction phase ethanol:water, 50:50 (v/v) at 50°C for 1 h, and the result, on average, was 62% higher than that achieved when using only acetone, ethanol, methanol, or water [21]. The mixture of an organic solvent and water can enhance sample hydration and promote the migration of phenolic compounds from plant tissues to solution through loosened cell walls [22]. Besides, the efficiency of the maceration process is also affected by other contributory factors such as the time of impregnation, the solvent to dry weight sample ratio, and the particle size of plant materials (**Table 1**).

2.1.2 Percolation

In this process, pulverized plant materials are packed in a percolator, and then extraction solvent is dropped continuously from the top toward the bottom to enable the solvent to percolate through plant materials [23]. This method is similar to maceration as it is also takes place based on molecular infusion; however, percolation is more efficient than maceration because it is the dynamic leaching method with the constant replacement of saturated solvent by fresher solvent [24]. Anwar et al. compared the antioxidant activity of *Eurycoma longifolia* L. extracts that are obtained by conducting different extraction methods including maceration, percolation, reflux, and soxhlet. Based on the results of the DPPH test, they indicated that the

Plant	Solvent	Time duration (h)	Liquid:solid (mL/g)	Particle size	TPC (mg GAE/g)	Ref.
Capparis spinosa L. leaves	Water	24 h	20: 1	< 850 μm mesh size	11.00 ± 1.54	[13]
Echinacea purpurea aerial parts	50% EtOH	24–72 h	—	< 850 μm mesh size	—	[14]
Propolis made by *Apis mellifera*	70% EtOH	24 h	50: 1	—	36.1–95.9	[15]
Anthyllis vulneraria L. aerial parts	Triphasic system: n-heptane/ EtOAc/ACN/ butanol/water (22:14:29:8:27)	24 h	10: 1	—	—	[16]
Eryngium planum and *Froriepia subpinnata* aerial parts	70% MeOH	24 h	10: 1	2–3 mm particle	44.75	[17]
Lysimachia nummularia L.	50% EtOH	24 h	10: 1	—	16.75–22.10	[18]

Table 1.
Some examples of the recent applications of maceration in the extraction of polyphenols.

antioxidant activity of percolation extraction was higher than the antioxidant activity of the maceration method and lower than that of reflux and soxhlet processes [25]. Another study by Shabani et al. mentioned that the total flavonoid content (TFC) of *Eryngium planum* L. extracts was in the order of percolation method > microwave extraction > maceration method > ultrasonic-assisted extraction, and the highest antioxidant capacity was observed in percolation extracts of *E. Planum* plant [17]. Percolation is suitable for substances that are prone to heat damage like phenolic compounds because it is often carried out at room temperature or gentle heating, though it is not an ideal method utilized for extracting substances from ingredients that lack organized structures [23]. In percolation, several factors impact the concentration of phenolic compounds in the extract, including solvent type, water content in organic solvent, extraction time, temperature, solvent to feed ratio, and particle size of ingredients (**Table 2**) [26, 28].

2.1.3 Decoction

Decoction is an extraction method utilized to extract compounds that are soluble in water and not prone to damage by high temperatures. In addition, decoction is the effective method to extract constituents from tough, fibrous plant parts such as barks or roots [29, 30] and is not suitable for plant materials that contain a high amount of starch, mucilage, gum, and pectin [23]. In this procedure, small pieces or powders of plant materials are boiled in water at 90–100°C for less than 1 hour and then filtered through filter paper (**Table 3**). Sometimes, the series of decoctions could be carried out to exhaustively extract compounds from medicinal plants rather than single decoction process. For instance, the extraction of phenolic compounds from *Combretum micranthum* was performed by placing whole or crushed leaves in constantly boiling water at 100°C for 20 minutes, and this procedure is repeated up to

Plant	Solvent	Time duration	Liquid:solid (mL/g)	Particle size	TPC (mg GAE/g)	Ref.
Peels of durian (*Durio zibethinus*)	MeOH	24 h	10: 1	~ 0.3 mm	250.39	[26]
Arctium lappa L. leaves	70% EtOH	1 week	—	—	1.034	[27]
Calophyllum inophyllum leaves	80% MeOH	48 h	10: 1	150 mesh	289.12	[28]
Eurycoma longifolia Jack. roots	96% EtOH Hot water	24 h	10: 1	—	—	[25]
Eryngium planum and *Froriepia subpinnata* aerial parts	70% MeOH	4 days	10: 1	—	44.44	[17]

Table 2.
Some examples of the recent applications of percolation in the extraction of polyphenols.

10 times to find out the optimal decoction conditions to use this plant in traditional medicine. The results showed that whereas the highest number of polyphenols (21.67%) was extracted from crushed leaves after three series of decoctions, the maximum content of polyphenols (16.17%) was obtained from whole leaves after six series of decoctions. This is because crushed leaves offer a larger exchange surface area, facilitating the penetration of the extraction solvent through plant tissues [35]. The main drawback of this method is that the high temperature could result in the destruction or the chemical transformation of thermolabile phenolic compounds, which leads to the reduction of antioxidant activities of decoction extracts compared to other methods [34, 36]. Rohmah et al. investigated that extended heating during decoction causes a decrease in the number of polyphenols from *Sesbania grandiflora* L. and their antioxidant activity [34]. In another example, the longer time of a decoction process remarkably impacted the content of rutin in *Matricaria chamomilla* L. extracts. Simultaneously, the content of quercetin-rutin's aglycone form significantly increased over time perhaps because of the hydrolyzation of glycosides. In addition, when conducting experiments with the mixtures of standard phenolic solutions, they indicated that some of which were unstable under the applied decoction process. Specifically, the concentrations of studied phenolic acids and flavonoids decreased following order: myricetin, quercetin, rutin, catechin, caffeic acid, chlorogenic acid, gallic acid, luteolin, *p*-coumaric acid, apigenin, hesperidin, and *p*-HBA [32].

2.1.4 Infusion

In hot infusion, the process is quite similar to decoction extraction as it takes place at high temperatures and uses water as the extraction solvent for a short period [1]. However, the difference between infusion and decoction is that plant materials are placed in boiling water and allowed to steep in the extraction phase for a certain period in the infusion process (**Table 3**) [37]. The extraction of phenolic compounds from *Sesbania grandiflora* L. was carried out by infusion method for 30minutes, while the decoction method was 15 minutes. The results indicated that the TFC value and TPC value of infusion extracts (347.2 ± 3.15 mg QE/g and 252.21 ± 3.64 mg GAE/g, respectively) were higher than those of decoction extracts (309.55 ± 2.29 mg QE/g and 215.74 ± 4.02 mg

Plant	Decoction	Infusion	Results	Ref.
Aerial parts of *Teucrium polium* L.	2 g of samples +98 mL water and being heated to 100°C and kept boiling for 15 min.	2 g of samples +98 mL boiling water and staying for 15 min.	While the decoction was found to be rich in flavonoids, the infusion was rich in phenolic acids.	[29]
Zingiber officinale rhizomes	4 g of powder +100 mL water and being heated to 100°C and kept boiling for 6 min.	4 g of powder +100 mL boiling water, staying for 10 min.	The TPC value of decoction extracts was about two times higher than those of infusion extracts.	[30]
Crataegus pinnatifida fruits	2 g of powder +100 mL water and being heated to 100°C and kept boiling for 10 min.	2 g of powder +100 mL boiling water, staying for 15 min.	The TPC value of decoction extracts was significantly higher than that of infusion extracts ($p < 0.05$).	[31]
Tea bags containing Matricaria chamomilla L. and *Hypericum perforatum*	2 g of powder +50 mL water and being heated to 100°C and kept boiling for 20 min.	2 g of powder +50 mL boiling water (~95°C), staying for 20 min.	The content of studied phenolics was lower in decoction in comparison with the infusion mode.	[32]
Zingiber officinale Roscoe var Roscoe leaves and rhizomes	Samples +250 mL water and being heated to 100°C and kept boiling for 10 min.	Samples +250 mL boiling water and staying for 10 min.	The TPC value and antioxidant activity of decoction extracts of leaves and rhizomes were higher than those of infusion extracts.	[33]
Sesbania grandiflora (L.) Pers. leaves	50 g of samples +100 mL water and being heated to 100°C and kept boiling for 30 min.	50 g of samples +100 mL boiling water and staying for 15 min.	The TPC and TFC values and antioxidant activity of infusion extracts were significantly higher than those of decoction extracts ($p < 0.05$).	[34]

Table 3.
Comparison of decoction and infusion methods in some recent researches.

GAE/g, respectively) [34]. However, in other cases, the efficiency of the infusion process was significantly lower than decoction extraction because the lower temperature of water in decoction was insufficient to promote the softening and decomposition of cell walls to release more insoluble-bound phenolics (**Table 3**) [31, 33]. For example, according to the results of Mahmudati et al., the phenolic content extracted by the infusion method was about two times lower than that of decoction extracts when conducting experiments in three ginger varieties (*Zingiber officinale* L.); however, there was no difference in antioxidant activities of extracts from two extraction methods [33].

2.1.5 Soxhlet

Soxhlet is considered the most effective conventional method for a continuous reflux extraction of active substances from plant materials. In this process, the extraction solvent is continuously refluxed and fresh solvent reaches materials in each cycle, which results in higher yields obtained because the saturation of solvent does not occur [38]. This extraction process is repeated usually each 10–15 min (4–6 cycles per hour), and continued until the substances in certain polarity are entirely extracted.

By using the solvent reflux and siphon principle, the total volume of the extraction phase is preserved during the Soxhlet extraction [37].

Soxhlet extraction requires less solvent and time than percolation or maceration when it comes to exhaustively extracting phenolic compounds from medicinal plants [9]. In addition, it does not require filtration after the extraction process as in other conventional methods and it also offers a good recovery when applied to both initial and bulk extractions [19]. However, the major drawbacks of this method are that the extracted substances are constantly heated at high temperatures during the extraction process leading to the decomposition of thermolabile compounds [39]. **Table 4** summarizes some recent applications of the soxhlet method in extracting phenolics from various plant matrices.

2.2 Unconventional extraction methods

2.2.1 Ultrasound-assisted extraction (UAE)

UAE technique is carried out by using acoustic waves in the kilohertz range spreading in a liquid medium. To extract active compounds from plant tissues, the frequencies applied in ultrasonication treatments often range from 20 to 100 kHz and from 10 to 1000 W/cm^2 in power density [45]. During UAE, the ultrasonic waves lead to the collapsing of cell walls, the reduction of particle sizes, and the destruction of plant matrices to enhance the extraction rate as well as facilitate the mass transfer of phenolics without the need for high temperature in the extraction process, enabling this method to minimize the breakdown of phenolic compounds and thermolabile substances in relation to other extraction techniques [46].

To optimize a UAE procedure, the effects of parameters including extraction solvent, liquid to solid ratio, extraction time, and temperature need to be evaluated [47] (**Table 5**). For example, Oroian et al. investigated the influence of four different

Plant	Solvent	Temperature (°C)	Extraction time (hours)	Liquid:solid (mL/g)	TPC (mg GAE/g)	Ref.
Zeravschania khorasanica aerial parts	80% EtOH	95 °C	6 – 8 h	40: 1	88.19 ± 1.99	[40]
Pimpinella anisum aerial parts	Water	100 °C	9 h	10: 1	3.28 ± 0.27	[41]
Lysimachia nummularia L.	70% EtOH	—	2 h	10: 1	38.10 ± 1.48	[18]
Arctium lappa L. roots *Polygonum aviculare* L.	50% EtOH	—	—	—	57.8 ± 0.03 25.5 ± 0.02	[42]
Anisophyllea disticha (Jack) Baill. stems	EtOH	—	12 h	5: 1	27.73 ± 4.10	[43]
Vigna radiata L. seed coats	95% EtOH	80°C	6 h	50: 1	—	[44]

Table 4.
Some examples of the recent applications of Soxhlet in the extraction of polyphenols.

Plant	Solvent	Temperature (°C)	Ultrasonic power/ frequency	Extraction time	Solvent:biomass (mL/g)	TPC (mg GAE/g)	Ref.
Solanum betaceum fruits	73% acetone	40°C	500 W	12 min	40: 1	15.07	[48]
Origanum vulgare ssp. hirtum leaves	60% EtOH	80°C	750 W	40min	20: 1	362.1 ± 1.8	[49]
Hibiscus sabdariffa L. calyxes	Water	52°C	50 Hz	58min	42: 1	5.25–10.58	[50]
Mango peels (*Mangifera indica* L.)	EtOH: acetone (60: 40)	25°C	400 W; 24 kHz	15 min	20: 1	128.8	[51]
Capsicum chinense leaves	50% MeOH	20–50°C	—	15 min	50: 1	24.39 ± 2.41	[52]
Propolis	80% EtOH	65°C	500 W; 20 kHz	25 min	10: 1	0.101 ± 0.272	[53]

Table 5.
Some examples of the recent applications of UAE in the extraction of polyphenols.

variables including ultrasonic amplitude, ethanol concentration, extraction temperature, and time on the efficiency of phenolic compounds extraction from *Apis mellifera* L. and a set of 29 experiments was obtained by randomly combining four factors based on the Box-Behnken design. The modified optimum conditions after considering the practical application were 100% amplitude of ultrasonic treatment, 70% ethanol, 58°C, and 30 min. Besides, the extraction yield under optimum conditions was 459.92 mg GAE/g of TPC and 220.62 mg QE/g of TFC [54]. In addition, by adjusting the ultrasonic power and frequency, the mass transfer could be enhanced leading to the increase in extraction yields, and the optimal effectiveness was obtained when operating at a lower frequency, specifically below 40 kHz [55]. The high ultrasound power and frequency (given in Watt and Kilohertz, respectively) usually exert a negative impact on the stability of phenolic compounds by generating a huge number of free radicals, especially hydroxyl radicals from the pyrolysis of water molecules within cavitation bubbles during the extraction process. The extraction yields of phenolic compounds from *Solanum betaceum* decreased when the amplitude of ultrasound treatment was raised from 50 to 70%, perhaps because the increase in this parameter beyond the optimum level promotes the severity of cavitation leading to the degradation of extracted compounds [48].

2.2.2 Microwave-assisted extraction (MAE)

This technique utilizes microwave energy, a form of electromagnetic radiation with a frequency range of 0.3–300 GHz, which produces heat by stimulating ion migration and dipole rotation in dielectric materials and polar compounds leading to the penetration of solvent into plant tissues [45]. Therefore, the effectiveness of the MAE process is profoundly dependent on the dielectric properties of the extraction phase and medicinal ingredients. To be specific, extraction solvents having permanent dipole moment with a high dielectric constant and a high dissipation factor as water, methanol, and ethanol are preferred since they strongly absorb microwave energy and offer a more homogenous heating in the extraction phase (**Table 6**) [1]. Besides, nonpolar solvents with a low dielectric constant like hexane and chloroform are microwave-transparent and may not be applicable in MAE procedures since they poorly absorb microwave energy leading to the inability to heat up [1]. In addition, water contained in plant tissues plays a key role in absorbing microwave energy and creating pockets of localized heating in ingredients, facilitating the collapse of plant cell walls and enhancing the release of phenolic compounds into the extraction phase (**Table 7**) [66].

In MAE, several parameters have crucial influences on the extraction yield, including solvents, temperature, microwave power, sample size, solid to liquid ratio, and extraction time (**Table 6**) [66]. In general, the increase in microwave power might result in shorter extraction time and higher extraction yields, especially for tough and hard plant parts like beans, roots, and stems (**Table 6**). However, a further increase in microwave power could be responsible for the degradation of extracted phenolic compounds as reported in the extraction of chlorophyll, carotenoid, and phenolic compounds from *Chlorella vulgaris.* The results indicated that the extraction yield decreased when the value of microwave power reached above 550 W [65].

2.2.3 Supercritical fluid extraction (SFE)

During the SFE process, pressurized fluids (mainly CO_2) are used as extraction solvents. In this technique, extraction solvents are in their supercritical state, which

Plant materials	Solvent	Temperature (°C)	Microwave power	Extraction time	Liquid:solid (mL/g)	TPC (mg GAE/g)	Ref.
Peach (*Prunus persica* L.) pulp	80% MeOH	58°C	500 W	20 min	16: 1	3.067± 0.027	[56]
Euphorbia hirta leaves	50% EtOH	40°C	400 W	2 min	12: 1	178.46 ± 0.01	[57]
Salvia fruticosa L. aerial parts	72% EtOH	40°C	950 W	15 min	30: 1	190.30 ± 6.62	[58]
Cocoa beans (*Theobroma cacao* L.)	73% MeOH	67°C	1500 W	56 min	20: 1	—	[59]
Cherry peels (*Prunus cerasus* L.)	80% EtOH	—	500 W	90s	300: 1	44.15	[60]

Table 6.
Some examples of the recent applications of MAE in the extraction of polyphenols.

Plant materials	Cosolvent	% of cosolvent	Temperature (°C)	Pressure	Extraction time	TPC (mg GAE/g)	Ref.
Rice husk (*Oryza sativa*)	50% EtOH	25%	60°C	30 MPa	20 min	1.29	[61]
Labisia pumila leaves	78% EtOH	16%	32°C	283 bar	4.5 h	—	[62]
Hippophae salicifolia leaves	EtOH	2.43%	47.5°C	25.1 MPa	1 h	84.31 ± 3.98	[63]
Zostera marina L.	EtOH	1%	60°C	250 bar	90 min	—	[64]
Anisophyllea disticha (Jack) Baill. Stems	EtOH	20%	50 °C	25 MPa	90 min	84.85 ± 0.35	[43]
Chlorella vulgaris	EtOH	10%	60°C	250 bar	3.3 h	17.30	[65]

Table 7.
Some examples of the recent applications of SFE in the extraction of polyphenols.

involves pressure and temperature above their critical points. These supercritical fluids have physicochemical properties of both liquid and gas states. To be specific, this fluid has a value of density similar to liquid (0.3–0.8 g/cm^3), a diffusion coefficient that is higher than liquid and lower than gas and a low viscosity similar to gas (10–4–10–3 g/s.cm) [67]. In other words, supercritical fluids have solvating properties that are similar to conventional extraction solvents but have higher transport capacity and lower viscosity, which facilitates fluid diffusion through plant tissues resulting in higher extraction yields compared to organic solvents [1, 23]. Besides, by adjusting pressure and temperature, the density of the supercritical fluid, which is related to the polarity property of the solvent, could be modified to improve the solubility of phenolic compounds in the extraction phase [62]. In this way, both the flexibility and selectivity of this technique are enhanced, enabling to selectively extract different types of bioactive compounds from medicinal plants.

Due to ideal properties like having low critical temperature (31.3°C) and moderate pressure (72.9 atm) as well as being able to be easily removed from extracts, carbon dioxide is commonly utilized in the SFE process [67, 68]. Generally, supercritical carbon dioxide (S-CO_2) is preferred for the extraction of nonpolar compounds because of its low polarity. However, some modifiers such as methanol, ethanol, or water should be added to S-CO_2 as cosolvents to extend the range of solvating strength enabling the improvement in the extraction yield when extracting polar substances like phenolic compounds [43, 61, 63]. Yerena-Prieto et al. determined the effects of different cosolvent combinations (25, 50, and 75% for water and ethanol, as well as a ternary mixture of CO_2-50%, ethanol-10%, and water-40%) on the recovery of phenolic compounds in the extracts of *Moringa oleifera* Lam. leaves. They concluded that the ratio of CO_2:H_2O 50:50 offered the highest value of TFC in the extracts (6.931 mg/g). Furthermore, this result also implied that the presence of water as cosolvents could enable water to interact with CO_2 to form carbonic acid. This acidified water might contribute to the breakdown of chemical bonds, such as glycosidic bonds in flavonoids and anthocyanins leading to the improvement in diffusion coefficient and the enhancement in release of phenolic compounds into the extraction phase [69].

2.2.4 Pressurized liquid extraction (PLE)

In the PLE process, pressurized solvents are utilized to enhance the transport capacity of solvents and promote mass transfer to improve extraction efficiency. In this extraction process, solvents are heated to above the solvent boiling point but below the critical point to decrease viscosity as well as to improve the kinetics of extraction, while keeping the solvents in liquid states by applying a high operating pressure [10, 68]. The enhancement of extraction kinetics helps to save time and reduce solvent consumption compared to conventional extractions. According to Supasatyankul et al., the results showed no significant difference between the value of TPC and TFC of the *Vigna radiata* L. extracts achieved by Soxhlet and PLE. While the Soxhlet method took 6 h for extraction, the PLE method took only 10 min [44].

Parameters such as solvents, temperature, and pressure should be modified to optimize the PSE process (**Table 8**). It is commonly known that the higher temperature would bring about higher extraction yields because the mass transfer is enhanced and the matrix interactions between phenolic compounds and samples obtained by hydrogen bonding, van der Waals forces, and dipole interactions are disrupted [23]. For instance, the TPC value of *Laurus nobilis* L. leaf extracts proportionally increased when increasing temperature from 80 to 160°C [71]. Furthermore, it is possible to modify the polarity of water by controlling the temperature and pressure at certain values, which are under critical points during the PLE process. In this way, extraction solvents that are not environmentally friendly could be replaced by subcritical water to extract low polar compounds [70]. In the PLE procedure, high pressure allows extraction solvents to maintain the liquid state at high temperatures, but the raw materials could be compacted when high pressure is applied during the extraction process.

Plant materials	Solvent	Temperature (°C)	Pressure	Extraction time	Extraction cycle	TPC (mg GAE/g)	Ref.
Fucus vesiculosus	Water	200 °C	10 bar	11–14 min	1	—	[70]
Laurus nobilis L. leaves	50% EtOH	150 °C	10.3 MPa	5 min	1	31.87–49.30	[71]
Vigna radiata L. seed coats	50% EtOH	160°C	1300 psi	10 min	1	55.27 ± 1.14	[44]
Arbutus unedo L. fruits	50% MeOH	120 °C	10 Mpa	10 min	2	183.26 ± 1.44	[72]
Eucalyptus intertexta leaves	44% EtOH	100°C	—	10 min	1	357.0	[73]
Brown seaweed (*Fucus vesiculosus*)	58.7% EtOH	137°C	—	4.68 min	1	369.0	[74]

Table 8.
Some examples of the recent applications of PLE in the extraction of polyphenols.

2.3 Discussion

According to the literature review, there are some research trends in the extraction of phenolic compounds from plant matrices:

- Comparing the efficiency of the extraction process between conventional and unconventional methods or among methods in the same conventional/unconventional groups based on some values like extraction yields, TPC, TFC, antioxidant activities of obtained extracts, or other biological activities, as well as investigating the degradation of phenolic compounds during different extraction methods [17, 32, 34, 48, 58, 65].

- Applying response surface methodology (RSM) based on central composite design (CCD), Box-Behnken designs (BBD), and statistical analysis (ANOVA) to investigate the effect of the independent variables (extraction solvents, time, liquid to solid ratio, etc.) on the extraction yields, TPC, or TFC as well as estimate the optimal extraction condition for the extraction process with the minimum number of experiments required [46, 49, 50, 63, 72].

- Scaling-up unconventional extraction methods from the lab to pilot/industrial scale by investigating some factors including instrumentation, batch/flow process, kinetics, economics, and energy consumption [44, 75–78]. Among unconventional techniques, SFE was the most common technique utilized for industrial applications with the highest scale-up investigations (60%), followed by UAE (15%) and MAE (14%) [79]. Besides, the combination of SFE and MAE, UAE, or PLE in pilot and semi-industrial scale has become more and more popular [75, 80].

- Conducting green extraction of phenolic compounds from plants by utilizing unconventional extraction methods in which the extraction solvent is water, deep eutectic solvents, or agrosolvents [70, 79, 81]. This new approach has brought up many benefits like safety, eco-friendliness, low cost, easy preparation, and wide availability. Deep eutectic solvents are a new type of ionic liquid analog. They are eutectics formed by two or more components, including an H-bond acceptor compound (HBA) and an H-bond donor compound (HBD) with a certain ratio of hydrogen bonds [82, 83]. These solvents have superior ability to solubilize polyphenols, so they have been widely utilized in the green extraction of phenolic compounds in recent years [84]. Furthermore, some tools like Analytical GREEnness (AGREE) could also be used to assess the greenness of the extraction procedures [85–88].

3. Analytical method

3.1 Chromatography

3.1.1 Liquid chromatography (LC)

3.1.1.1 Columns

HPLC is one of the most prevalent analytical methods and is widely utilized to quantify and identify phenolic compounds along with LC-MS and GC-MS [10].

The principle of HPLC is based on the comparison between unknown compounds and reference standards to conduct quantitative and qualitative analytical measurements. Furthermore, a higher resolution form of HPLC is ultra high-performance liquid chromatography (UHPLC) or ultra-performance liquid chromatography (UPLC) characterized by smaller particle size (typically <2 μm). This technique has been increasingly prevalent due to its higher theoretical plate count, leading to the improvement in separation efficiency [89].

There is a great range of uniformity columns that could be selected for HPLC to satisfy intended selectivity such as butyl, octyl, phenyl, cyano, or fluorinated columns; among them, reversed phase with the octadecyl group (RP-C18) is the most popular option when selecting a stationary phase to determine phenolic compounds in plant matrices (**Table 9**). Besides, an HILIC column could be used to separate hydrophilic phenolic compounds from *Matricaria chamomilla* L. extracts as reported by Pyrzinska et al. [32]. From the literature review, the commonly chosen RP-C18 columns often have internal diameter (i.d.) ranging from 2.1 to 5 mm for HPLC (the most common is 4.6 mm) and smaller i.d. ranging from 1.1 to 2.1 mm for UPLC. Besides, the particle size in these columns, in most cases, ranges from 2.6 to 5.0 μm for HPLC and 1.6 to 2.0 μm for UPLC. In addition, the mobile phase is usually the mixture of water and organic solvents like methanol and acetonitrile, and an organic acid like acetic acid, formic acid, or phosphoric acid is often added to acidify the mobile phase (**Table 9**).

3.1.1.2 Detectors

To determine phenolic acids and flavonoids, HPLC coupled with UV-Vis or photodiode array (PDA) detector is predominant among other detectors like fluorometric detection (FLD) and evaporate light scattering detector (ELSD) due to its popularity, robustness, and adequate sensitivity. Some parameters of detectors should be taken into consideration when setting up a method including detector time-constants, data-system sampling rate, and the stability of temperature to minimize noise and baseline drift, resulting in the improvement of the signal-to-noise ratio [91].

As shown in **Table 9**, most phenolic acids are measured by UV-Vis–based detectors at 220–290 nm because they have the benzoic acid carbon framework, and flavonoids could be determined at two ranges including 250–270 nm and 340–370 nm. By coupling HPLC with PDA, the peak purity would be easily checked and researchers can conclude more certainly about peak homogeneity. In addition, the UV spectrum of each phenolic compound can be obtained when scanning from 200 to 400 nm during the HPLC process [91]. These spectrums, when being compared to the reference standard spectrum in available databases, could provide information to identify structures of phenolic compounds in plant samples [92, 93]. Besides, in the last few decades, FLD has emerged as an alternative and complementary analytical tool to quantify phenolic compounds other than UV-Vis and MS detector. For example, the phenolic and polyphenolic fingerprints of paprika from five European regions were obtained by utilizing HPLC-FLD with an excitation wavelength of 310 nm and an emission wavelength of 380nm [94]. In another example, the distribution of phenolic substances (gallic acid, caffeic acid, rutin, rosmarinic acid, and quercetin) in each solvent phase (apolar, intermediate, and polar) was determined based on measuring area under the curve (AUC) value, obtained by using HPLC coupled with ELSD [16].

Plant materials	Extraction process	LC condition	Detector condition	Quantified phenolics	Ref.
Dried leaves of green tea (*Camellia sinensis* L.)	SFE with SC-CO_2 (80°C, 30% EtOH: H2O 80:20), for 15 min, at 15 MPa	UHPLC-PDA Cortecs C18 (100 × 3.0 mm; 2.7 μm); mobile phase: A-0.1% FA in water, B-0.1% FA in MeOH, C-ACN, gradient; 1 mL/min; analysis time: 23 min	Wavelength monitoring: 210 nm	(−)-Epicatechin gallate, (+)-catechin, (−)-epigallocatechin, (−)-epicatechin, (−)-epigallocatechin gallate, caffeine, (+)-gallocatechin	[90]
Aerial parts of *Salvia fruticosa* L.	UAE (20 kHz) with 68% EtOH, solvent to solid ratio of 10:1 mL/g, for 10 min, at 47°C MAE (600 W) with 72% EtOH, solvent to solid ratio of 30:1 mL/g, for 15 min, at 40°C	LC-PDA-ESI-MS Poroshell 120 EC-C18 (150 × 4.6 mm, 4 μm); mobile phase: 0.1% FA in water and ACN, gradient; 0.5 mL/min; analysis time: 45 min	Wavelength monitoring: scanning from 200 to 400 nm Single quadrupole combined with ESI: negative ion mode Acquisition in SIM mode	Rosmarinic acid, carnosol, carnosic acid	[58]
Peach (*Prunus persica* L.) pulp	UAE (600 W) with MeOH 80%, solvent to solid ratio of 35:1 mL/g at 35°C, for 15 min MAE (50 W) with MeOH 80%, solvent to solid ratio of 16:1 mL/g, at 58°C, for 20 min	HPLC-ESI-QqQ-MS/MS Zorbax Eclipse Plus C-18 (50 × 2.1 mm; 3.5 μm); mobile phase: A-0.2% FA in water and B-0.1% FA in ACN, gradient; 0.3 mL/min; analysis time: 15 min	ESI: negative ion mode, curtain gas 10 psi, ion spray needle voltage of 4.5 kV, source temperature of 600°C, nebulizer gas pressure of 50 psi, heater gas pressure of 50 psi Acquisition in MRM mode	Chlorogenic acid, naringenin	[56]
Leaves of *Hippophae salicifolia*	SFE with SC-CO_2 (25.13 MPa, 47.53°C, 14.47 g/min, EtOH 2.43%) for 2 h	HPLC-UV-Vis RP-C18 (250 × 4.6 mm; 5 μm); mobile phase: A-0.1% phosphoric acid in water and B-1% phosphoric acid in ACN, isocratic; 1 mL/min for phenolic acids A-water, B-MeOH, C-ACN each with 1% AA, isocratic; 0.5 mL/min for flavonoids	Wavelength monitoring: 280 nm for gallic acid, 270 nm for vanillic acid, 296 nm for caffeic and ferulic acids, 310 nm for p-coumaric acid, 368 nm for quercetin, myricetin, kaempferol, and rutin	Gallic acid, caffeic acid, ferulic acid, vanillic acid, p-coumaric acid, quercetin, myricetin, kaempferol, and rutin	[63]

Table 9.
Some examples of the recent applications of HPLC/UHPLC in the determination of polyphenols.

3.1.1.3 Quantification

HPLC coupled with UV-Vis based detector (like PDA) is the most prevalent tool in the quantification of phenolic compounds in plant matrices. However, the complexity of plant samples and the small amount of phenolic compounds in plants are sometimes the main challenges of PDA detectors. Furthermore, polyphenols bonded to sugar groups that are not UV-active, leading to many difficulties in identifying phenolic compounds by UV-Vis based detectors or even in predicting compounds that potentially exist in samples [4, 10]. Therefore, in some cases, an LC system coupled with a mass spectrometer (LC-MS) or a tandem mass spectrometer (LC-MS/MS) is a more effective analytical tool for identifying and quantifying phenolic compounds in complex matrices because of its higher sensitivity and better selectivity [32, 59]. To enhance the capacity of these systems, the hyphenation of UPLC system and MS system is preferred to HPLC. For instance, Irakli et al. quantified main phenolic substances (rosmarinic acid, gallic acid, catechin, carnosol, and carnosic acid) in *Salvia fruticosa* L. extracts by using the LC system equipped with a PDA detector and a single quadrupole mass spectrometer. The composition of the mobile phase was aqueous formic acid (0.1%, *v*/*v*) and acetonitrile with a gradient elution [58]. Some other examples are summarized in **Table 9** and the application of mass spectrometers will be mentioned in detail in Section 3.3 "Mass spectroscopic measurements".

3.1.1.4 Chromatographic fingerprints

It is widely known that the quality of medicinal plants is related to the composition of active substances, which often varies from batch to batch, depending on many factors, for example, phenolic compounds accumulated in wild *Physalis* species (*P. angulata, P. hederifolia, P. solanaceae, P. patula and P. subulata*) change depending on species, plant genotypes and chemotypes, morphological parts of the plant, growth stages, time of harvest, eco-geographical growth conditions, and storage conditions [95–97]. Therefore, the chromatographic fingerprint profiles provide information about the variations of phenolic compounds from a certain plant in an integrated manner. This analysis method could help to discriminate a plant from other closely related species or other cultivars, to authenticate its geographical origin or to distinguish authentic plant materials from substitutes and adulterans based on the statistical evaluation of the chemical differences and similarities in the HPLC chromatograms of samples [94, 98, 99]. In addition, fingerprint technology for identification of medicinal plants is the favored method in the framework of the ISO-standardization of the "Quality and safety of traditional Chinese medicine" [100]. Besides, this technique is also internationally accepted as a method for the quality control of herbal drugs and preparations and is considered as part of the quality proof of plants other than the DNA fingerprint [10, 100]. Some chemometric modeling methods including principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), heatmap analysis, hierarchical cluster analysis (HCA), and similarity analysis (SA) are applied in combination with fingerprint analysis to further process the chromatographic results, classifying samples according to a certain characteristic (like different species, cultivars, geographical origins, or growth stages) and proposing markers that could be used for the evaluation and quality control of medicinal plants (**Table 10**) [101, 104–106].

Plant materials	Extraction process	LC condition	Characteristic fingerprint peaks	Chemometric Analysis	Ref.
Walnuts (*Juglans regia* L.).	UAE with 0.05% TFA in MeOH 60%, at 30 °C, 10 min	HPLC-PDA C18 Fortis UniverSil column (250× 4.6 mm, 5 μm); mobile phase: 1% acetic acid solution and ACN; analysis time: 60 min Wavelength monitoring: 280 nm	Catechin, diosmin, epigallocatechin gallate, gallocatechin gallate, kaempferol, myricetin, myricitrin, quercetin-3-o-glucoside, rutin, vanillin and sinapic, syringic, vanillic, caffeic, ferulic, gallic, rosmarinic, and p-coumaric acids	PLS-DA	[101]
Dried leaves of *Akebia quinata*.	UAE (280 W) with 70% EtOH, solvent to sample ratio of 80:1 mL/g, 80 min	HPLC-PDA RStech HECTOR-M C18 column (250 × 4.6 mm, 5 μm); mobile phase: 0.1% formic acid in water and ACN; 0.5 mL/min; analysis time: 100 min Wavelength monitoring: 300 nm	Neochlorogenic acid, nicotiflorin, chlorogenic acid, cryptochlorogenic acid, 5-*O*-*p*-coumaroylquinic acid (peak 5), 5-*O*-feruloylquinic acid, rutin, quercetin-3-*O*-glucoside, isochlorogenic acid A, astragalin, and isochlorogenic acid C	HCA, PCA, and PLS-DA	[102]
Wine spirits The casks used to age the brandies were made of 3 different oak woods (Quercus species): *Q. alba, Q. robur, and Q. petraea*.	—	UPLC-PDA Acquity UPLC C18 BEH (100 × 2.1 mm, 1.7 μm); mobile phase: A (3% ACN, 2% acetic acid, 95% water) and B (85% ACN, 2% acetic acid, 13% water); 0.7 mL/min, analysis time: 12.5 min Wavelength monitoring: 280 nm	Gallic acid, 5-hydroxymethylfurfural, furfural; vanillic acid, *p*-hydroxybenzaldehyde, 5-hydroxymethylfurfural, syringic acid, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde	PCA and PLS-DA	[103]
Dendrobium species *(D. nobile, D. chrysotoxum, D. huoshanense, D. fimbriatum)*.	Reflux three times with 90% MeOH, solvent to sample ratio of 60:1 mL/g, 80°C, 1 h Using CNWBOND LC-C18 SPE to remove interferences	HPLC-PDA Zorbax Eclipse XDB-C18 (150 × 4.6 mm, 5 μm); mobile phase: water and ACN; 1.0 mL/min; analysis time: 35 min Wavelength monitoring: 220 nm	Isoschaftoside, coelonin, nudol, gigantol, erianin, 2,4,7-trihydroxy-9,10 dihydrophenanthrene, dihydroresveratrol, schaftoside, 4-Methoxy-2,5-phenanthrenediol, tristin 3-hydroxy-3,4,5-trimethoxybibenzy	Calculating correlation coefficients by cosine angle method	[98]

Table 10.
Some examples of the recent applications of chromatography fingerprints in the determination of polyphenols.

3.1.2 Gas chromatography (GC)

Apart from LC, GC is also a useful tool to analyze both phenolic acids and flavonoids in plant matrices. This technique offers better chromatographic separation by using capillary columns and consumes a much smaller number of organic solvents

when compared to LC [107]. Moreover, GC-MS provides better analysis of substances in complicated matrices with fewer matrix effects than LC-MS. This method also reduces ionization suppression and adduct formation and has many MS spectrum libraries from various resources for structure elucidation [108]. However, when utilizing GC to determine phenolic compounds, the main issue is the low volatility and thermal sensitivity of these compounds.

3.1.2.1 Derivatization

It is necessary to derivatize phenolic compounds before being analyzed by GC. The derivatization step could help reduce their polarity while improving volatility and thermal stability. There are many reagents that could be utilized to etherize or estherize phenolic hydroxyl groups of phenolics, but silylation is the most common strategy. In this process, hydrogens from hydroxyl groups are replaced by silyl groups of silanizing agents, forming volatile molecules that have greater thermal resistance [109]. For the silylation of phenolic compounds, several commercially available signalizing reagents like N,O-bis-(trimethylsilyl)acetamide (BSA), N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA), N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA), and N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) could be applied, and the most commonly used reagents are trimethylsilyl (TMS) derivatives including BSA, MSTFA, and BSTFA [110, 111].

The advantages of the silylation approach are that the reaction is simple, silylating reagents usually have high reactivity, and by-products generated from these reactions are extremely volatile and often elute earlier than the main products. In addition, the mass spectral information of TMS derivatives is provided by searchable databases and libraries such as the Golm Metabolome Database (GMD) and the MassBank MS Database [109]. However, there are some drawbacks to this procedure. One issue is that it takes a long derivatization time of 2–4 h, which directly reduces the analytical frequency. Additionally, it requires a high temperature to stimulate the reaction rate and an additional dry step since these reactions are only carried out in a water-free environment (**Table 11**). In addition, multiple derivative peaks of one phenolic compound with different degrees of TMS silylation might be detected, such as quercetin (5TMS), quercetin (4TMS), quercetin (3TMS), etc. This is particularly true for those substances carrying a high number of hydroxyl groups like flavonoids and phenolic acids. In recent years, the derivatization with alkyl chloroformates has also been employed instead of TMS reagents to some extent. In contrast to the silylation, this derivatization process could be carried on quickly in aqueous media at room temperature. However, the MS information of these ethoxycarbonyl derivatives is not sufficiently represented in available spectra libraries for GC-MS platforms based on electron ionization (EI) [108].

3.1.2.2 Chromatographic conditions

Based on the literature review, it can be concluded that fused silica capillary columns are the most commonly used for analyzing phenolic compounds after the derivatization process. The column lengths are 30 m, with inner dimensions ranging from 0.25 to 0.32 mm and a film thickness typically ranging from 0.1 to 0.25 μm (**Table 11**). The most commonly used stationary phase is a mixture of 5% phenyl and 95% dimethylsiloxane, which is a low-polarity phase that tends to separate compounds based primarily on their boiling points [111].

Samples/targeted analytes	Extraction process	Cleanup and derivatization steps	GC condition	Phenolic compounds	Ref.
Microbial-generated phenolic metabolites derived from grape flavanols	—	Derivatization with HMDS and TMCS in pyridine with the ratio of 3:1:9 at 70°C for 4 h HMDS: hexamethyldisilazane; TMCS: trimethylchlorosilane.	GC-QqQ-MS Shimadzu RP-5SilMS capillary column (30.0 m × 0.25 mm; 0.25 μm) EI ion source Acquisition: MRM mode	Gallic, caffeic, *trans*-p-coumaric, dihydrocoumaric, 3-(3,4-dihydroxyphenyl) propionic, 3,4-dihydroxy benzoic, hippuric, homovanillic, 3-hydroxybenzoic, 3-(3-hydroxyphenyl) propionic, ferulic, 3, 4-dihydroxyphenylacetic, 5-(4-hydroxyphenyl) valeric acids, catechin, and epicatechin	[107]
12 different commercial fruit juices	Liquid-liquid extraction (LLE) with hexane after alkalization samples by $NaHCO_3$ (pH 9)	Derivatization with ethyl chloroformate (ECF) in EtOH, at room temperature for 2 min; pyridine is the catalyst.	GC-QqQ-MS Agilent HP5ms fused-silica capillary column (30 m × 0.25 mm; 0.25 μm) EI ion source Acquisition: full scan (m/z from 50 to 650) and SIM modes	Benzoic, trans-cinnamic, 3-dimethylaminobenzoic, 3,4-dimethoxybenzoic, 2-hydroxybenzyl alcohol, vanillic, phloretic, homovanillic, p-coumaric, syringic, gentisic, ferulic, isoferulic, caffeic, dihydrocaffeic and homoprotocatechuic acids, resorcinol, and tyrosol	[108]
Wine samples	LLE with EtOAc for 2 times, stirring time 120 s	Derivatization with HMDS in pyridine for 180 s, with 40% of the microwave oven power. HMDS: hexamethyldisilazane.	GC-FID The capillary column (30 m × 0.25 mm; 0.1 μm) with 5% phenyl FID: operating at 320°C, hydrogen gas 40 mL/min, synthetic air: 400 mL/min	Gallic acid, caffeic acid, resveratrol, (−)-epicatechin, and (+)-catechin.	[112]

Table 11.
Some examples of the recent applications of GC in the determination of polyphenols.

According to Marsol-Vall et al., the retention times of TMS derivatives of phenolic compounds on a low-polarity column were found to increase with the number of TMS groups. To be specific, the retention time was observed to increase in the following order: aglycone < monoglycoside < diglycoside compounds when they have the same aglycone. In addition, the retention order of TMS derivatives with the same substituents was flavan-3-ol < chalcone < flavonone < isoflavone < flavonol < flavone [108].

3.1.2.3 Detectors

In the past, the flame ionization detector (FID) was the most commonly used detector since its introduction in 1958. This was due to its robustness, large dynamic linear range, low detection limits, and (nearly) universal behavior for organic compounds [108]. However, in recent years, the use of mass spectrometry (MS) as a "mass selective detector" in combination with GC has become increasingly widespread. For GC-MS analysis of phenolic compounds, EI is the preferred technique to generate ions from neutral molecules, and the use of this ionization source offers a mass spectrum with more fragments, facilitating the identification purposes [107–109, 113].

3.2 Mass spectroscopic measurements

As mentioned above, liquid chromatography (LC) coupled with mass spectrometry (MS) is a hyphenated technique that is now widely used to determine phenolic compounds in plant matrices, either coupling with low-resolution (LC-LRMS) or high-resolution instruments (LC-HRMS). Based on the advancement of these instruments gained in recent years, two main approaches are applied in the analysis of phenolic compounds in complex matrices, as well as in evaluating the bioactivity and nutraceutical potential of plants.

3.2.1 Targeted approaches

The targeted approach focuses on quantifying and identifying preestablished compounds by using LC coupled with low-resolution mass spectrometry such as single-quadrupole, tandem mass spectrometry (LC-MS/MS) using ion trap spectrometers (IT), or more often triple-quadrupole (QqQ) mass analyzers [5]. In this approach, chromatographic behavior (such as retention time, elution order, peak area, and peak height) and fragmentation behavior (like theoretical mass, m/z value of precursor, and product ions) of the reference standards and target compounds are compared to confirm the identity of phenolic compounds and quantify them [29]. Therefore, this approach requires the availability of reference standards for quantitative and confirmatory purposes. Besides, to obtain the chemical profile of a certain plant, the targeted compound finding is applied parallel to the nontargeted strategy based on the information about preestablished compounds found in this plant obtained from the literature review [110, 114–116]. Given the limited availability of phenolic compound standards, targeted approaches could be applied to determine a limited number of substances.

Besides, the most common ionization technique in LC-MS/MS for polyphenol analysis is electrospray ionization (ESI). Most phenolic compounds are often ionized with high sensitivity in negative mode, generating the deprotonated molecule [M-H]-, while anthocyanins could be ionized in both positive and negative modes [117].

Some parameters of the ion source and collision cell such as capillary voltages, cone voltages, source temperature, desolvation temperature, cone gas flow, and collision energy should be optimized by using reference standards to ensure stable and adequate detection of phenolic compounds [115, 116] (**Table 9**). For example, Lou et al. carried out a research to investigate phenolic compounds and antioxidant activity of Chinese organic hawthorn berry infusion and decoction (*Crataegus pinnatifida*) extracts, and then they assessed the digestive fate of phenolics by in vitro gastrointestinal model (VGIM). An HPLC-MS/MS system with an RP-C18 column (100 mm × 3.0 mm, 3.5 μm) and the mobile phase combining 0.5% acid acetic in water (A) and 100% acetonitrile (B) was utilized in gradient elution to determine 9 phenolic acids, 6 flavonoids, and 2 procyanidins. These phenolics were identified by using a tandem quadrupole mass spectrometer equipped with an electrospray ionization source (ESI) in negative mode, and the acquisition mode was MRM [31].

3.2.2 Nontargeted approaches

While targeted approaches offer the adequate selectivity and sensitivity required for the determination of phenolics in complex matrices, nontargeted approaches provide greater scope than targeted ones. The major aim of the nontargeted strategy is to obtain as much chemical information as possible from samples and to focus on identifying both known and unknown compounds whose reference standards might not be available.

Although all LC-MS systems can perform full spectral acquisition mode to obtain a full-scan mass spectrum of analytes, not all can provide sufficient sensitivity and selectivity in this mode for nontargeted screening. Therefore, HRMSs, especially Q-TOF-MS/MS instruments, are preferred because they offer accurate mass measurement and enhanced sensitivity in full-scan modes, enabling greater capability to identify unknown compounds than QqQ-MS and Ion trap-MS [7]. Q-TOF-MS/MS has a high sensitivity for phenolics detection, offers high mass resolution in broad mass range ($m/z < 10{,}000$) with resolving power ranging from 10,000 to 40,000, and provides high mass accuracy (< 3 ppm) with fast scanning speed (10–100 Hz) for both precursor and product ions [118]. When coupled with LC, this technique offers a higher performance and chromatographic resolution than other HRMS techniques due to the higher scanning rates of Q-TOF-MS/MS. Moreover, LC separation reduces the complexity of plant samples, leading to a decrease in the number of charged phenolic substances simultaneously entering the mass spectrometer. Consequently, the phenolic loss and the competition for charge would be minimized, resulting in the improvement of ionization performance. This method provides qualitative information based on molecular weight, characteristic fragmentation pathways, and retention behavior of phenolic compounds [119, 120]. For phenolic compounds' analysis, the negative ionization mode of ESI offers more characteristic fragments with stable intensity than the positive mode [121]. Apart from ion source parameters, some factors related to mass analyzers like resolution, scan rate, and mass acquisition interval should be optimized when utilizing LC-HRMS/MS.

3.2.2.1 Fragmentation behavior of phenolic acids

After being ionized in negative mode, all phenolic acids have deprotonated sites in the carboxylic group owing to the high acidity of this group. From these ions,

the loss of the carboxylic fraction (CO_2, 44 Da) is commonly observed. Besides, if phenolic acids have one or more methoxy groups like ferulic acid and synaptic acid, the first cleavage would occur through the •CH3 (15 Da) elimination, followed by the elimination of CO_2 (44 Da) [122]. For phenolic glycosides like gallyl glucose and caffeoyl glucose, the MS2 fragmentation showed the product ions formed by the loss of hexosyl moiety (162 Da) and the further loss of CO_2 (44 Da) or H_2O (18 Da) [123].

3.2.2.2 Fragmentation behavior of flavonoids

For flavonoid aglycones, the retro-Diels-Alder (RDA) reaction path combined with the loss of small molecules or radicals is the main fragment pathway (**Figure 1**). The RDA cleavage occurring in the C-ring forms two fragment ions containing A- and B- rings, which are denoted as $^{i,j}A^{\pm}$ and $^{i,j}B^{\pm}$ ions [125]. These ions with different m/z values provide information on the number and type of substituents located in A- and B-rings. This cleavage might occur in the C-ring at 1/3, 1/2, 0/2, 0/3, 0/4, or 2/4, and the fragmentation pathway mainly depends on the substitution pattern and the class of flavonoids [124]. For example, the most useful RDA fragmentation of flavones, flavanones, and flavonols involves the cleavage at positions between bonds 1/3 and 1/2 forming $^{1,3}A^{\pm}$, $^{1,3}B^{\pm}$ and $^{1,2}A^{\pm}$, $^{1,2}B^{\pm}$ products ion, respectively [126]. Besides, the $^{1,3}A^{\pm}$ ion, which is observed for all flavonoid groups, is the fragment most readily formed, and it is observed that $^{1,3}A^{\pm}$ and $^{1,2}A^{\pm}$ ions are the most prominent product ions for luteolin (flavone), naringenin (flavanone), and kaempferol (flavonol) [7]. When substituting with C′-2 hydroxyl, chalcone, which does not have the C-ring, is transformed into flavanone and might be fragmented following the RDA pathway [127]. Moreover, hydroxyl-substituent groups and their possible position in the aglycone could be identified by the loss of small molecules or radicals $[M \pm H]^{\pm}$ ions like H_2O (18 Da), CO (28 Da), CO_2 (44 Da), C_2H_2O (42 Da), and C_3O_2 (68 Da). These eliminations occurred in some flavonoid groups and their implications are summarized in **Table 12**.

For flavonoid glycosyl derivatives, three forms including C–, O–, and C,O–glycoside forms could be differentiated based on the difference between the corresponding X_j, Y_j, and Z_j fragment ions (**Figure 1**). Besides, the glycoside position and interglycosidic linkages ($1 \rightarrow 6$ or $1 \rightarrow 2$) could be determined by comparing the intensity of Y_1, Y_0, and Z_1 fragment ions (**Table 12**).

Thus, the mass spectra obtained from the LC-ESI-HRMS^n instrument provide structural information to determine the chemical structures of phenolics by assigning some characteristic product ions.

3.2.2.3 Data acquisition modes

Apart from conventional acquisition modes available in LRMS (full scan, SIM, SRM, and MRM), the new approaches including data-dependent acquisition (DDA) and independent data acquisition (DIA) have recently been applied in the analysis of phenolics by HRMS instruments. In DDA approaches, the full scan mode is applied in MS^1, and precursor ions for MS^2 or TOF scan are selected based on the predefined threshold value and by using inclusion – exclusion lists. By selecting precursor ions for further fragmentation, DDA has the potential to improve metabolite assignment and provide cleaner and more selective spectra compared to DIA approaches [139]. For example, in the research conducted by Zuo et al., the five most intense precursor

	Phenomena	Occur in (groups/rings)	Indication/implication	Ref.
Aglycones	The loss of H_2O (18 Da)	Flavones	An ortho-hydroxyl group	[128]
	The loss of C_3O_2 (68 Da)	Flavones	A 5–7-dihydroxy in A-ring	[121]
	The loss of CO (28 Da) and CO_2 (44 Da)	C-ring	Occurs at C4 in the C-ring	[129]
	The loss of C_2H_2O (42 Da)	B-ring	A 4′-hydroxy in B-ring	[130]
		C-ring	Leading to cyclization	
	The loss of C_4H_8 (56 Da)	A- or B-ring	Prenylated flavonoids	[131]
	The loss of C_5H_8 (68 Da)			
	The loss of C_9H_{16} (124 Da)			
	The loss of $C_{10}H_{16}$ (136 Da)			
	The loss of CH_3• (15 Da)	A- or B-ring	Methoxylated flavonoids	[132]
Glycosylated flavonoids	The loss of $C_4H_8O_4$ (120 Da)	C-glycosides	C-hexose	[133]
	The loss of $C_3H_6O_3$ (90 Da)			
	The loss of $C_2H_4O_2$ (60 Da)	C-glycosides	C-pentose	
	The loss of $C_3H_6O_3$ (90 Da)			
	The loss of $C_3H_6O_2$ (74 Da)	C-glycosides	C-deoxyhexose	
	The loss of $C_4H_8O_3$ (104 Da)			
	The presence of $[^{0,2}X_0 - H_2O]^+$, $[^{0,2}X_0 - CHO]^+$, $[^{0,2}X_0 - H_2O - CO]^+$ ions	C-glycosides	6-C-glycoside	[134]
	High abundance of $[^{0,2}X_0 - CHO]^+$ ions	C-glycosides	8-C-glycoside	
	The loss of $C_6H_{10}O_5$ (162 Da)	O-glycosides	O-hexose	[135]
	The loss of $C_5H_8O_4$ (132 Da)		O-pentose	
	The loss of $C_6H_{10}O_4$ (146 Da)		O-deoxyhexose	
	High abundance of $(Y_0 - H)^{-\bullet}$ ions		3-O-glycoside	[136]
	High abundance of $^{1,3}B^-$ and $^{1,3}A^-$ ions		7-O- or 4′-O-glycosides	
	Y_1^- ion is the base peak in the spectra		di-O-glycosides	[137]
	Y_0^- ion is the base peak in the spectra		O-diglycosides	
	Z_1^- ion is the base peak in the spectra	C,O-glycosides	C,O-diglycosides	[138]
	Y_0^- ion exhibits the highest abundance		di-C,O-glycosides	
	The abundance of Y_0^+ ion > Y_1^+ ion	O-diglycosides	1 → 2 linkages	[124]
	The abundance of Y_0^+ ion < Y_1^+ ion		1 → 6 linkages	
	The presence of $^{0,2}X^-$ fragments	C,O-diglycosides	1 → 2 linkages	
	The loss of an O-glycosyl unit and the presence of $[Y_1 - ^{0,2}X]^-$		1 → 3, 1 → 4, or 1 → 6 linkages	

Table 12.
Some MS fragmentation behaviors of phenolic compounds.

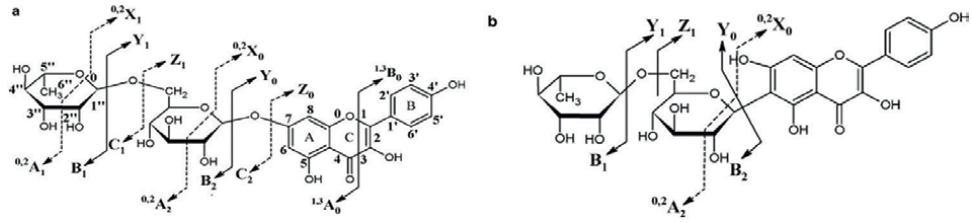

Figure 1.
Fragment nomenclature commonly applied for (a) O-glycosides and (b) C,O- and C-glycosides [124].

ions when the intensity exceeded 1000 detector counts (threshold values) were automatically applied to do MS/MS fragmentation [140]. Aita et al. added an exclusion list containing the most intense ions detected in the blank sample to the mass spectrometric method, and these ions would never be selected as precursor ions for the MS^2 scan, even if they exceeded the defined threshold. This list excludes background interferences and minimizes the noise in the mass spectrometry [141].

The main disadvantage of DDA is the risk of missing precursor ions since these ions are not selected for fragmentation in MS^2 if they do not meet the specified threshold and user-guided criteria. Besides, the drawbacks of DIA come from the missing preselection, causing impure mass spectra and the loss of direct links between specific precursor ions and their corresponding fragment ions. As both DDA and DIA approaches have advantages and disadvantages, some studies were conducted to compare and evaluate the performance or apply a combination of DDA and DIA approaches in phenolic compound analysis. For instance, Shah et al. utilized LC-ESI-Q-TOF/MS with the combination of DDA and DIA modes to obtain the MS data of *Tribulus terrestris* L. extracts. The results indicated that 95 and 77 metabolites were identified from plant fruit samples in positive and negative ionization modes including flavonoids, alkaloids, terpenoids, and saponins. In addition, the data obtained by DIA was better than DDA and can be used for the quantification of both MS^1 and MS^2 values [142]. In another example, a total of 131 bioactive compounds, of which 31 were flavonoids, were identified from *Aster tataricus* rhizome samples by UHPLC-Q-TOF-MS with DDA and DIA approaches. Among 131 compounds, 120 compounds were identified by DDA, while DIA could detect all 131 compounds [114].

3.2.2.4 Databases

Datasets obtained from a nontargeted screening process by using LC-HRMS systems often contain information about retention/migration time, mass-to-charge ratio, and intensity. To manage and analyze a large amount of these raw datasets, it is essential to utilize appropriate data processing software and suitable databases.

Based on molecular fragments obtained from mass spectra, the molecular formulae of phenolic compounds could be calculated with a mass accuracy of <3 ppm and then screened against databases such as ChemSpider, PubChem, DrugBank, CompTox, SciFinder, METLIN, or the polyphenol database (http://phenolexplorer.eu/). Besides, other databases like MassBank, HMDB, Metlin, NIST, and GNPS could provide information about MS/MS mass spectra (**Table 13**)

Plant materials	Compounds	Techniques	Results	Data processing approach	Ref.
Whole annual branches with leaves and flowers of *Spiraea hypericifolia*	Total 47 compounds 23 flavonoids 6 phenolic acids	LC-ESI-Q-exactive HF Orbitrap MS	A chromatographic profile of the aqueous-ethanol extract of *S. hypericifolia* were obtained.	mzCloud database ChemSpider database	[119]
Aerial parts of selected *Crepis* species (C. *commutata, C. dioscoridis, C. foetida, C. heldreichiana, C. incana, C. rubra, C. crocifolia*)	Total 52 compounds 18 flavonoids 15 phenolic acids	LC-Q-TOF-MS/MS	The main constituents of *Crepis* spp. were confirmed to be caffeoyl tartaric acid derivatives.	In-house database (approximately 200 compounds described previously in *Crepis* species)	[120]
Auricularia cornea	2 phenolic acids 9 flavonoids	LC-Q-TOF-MS/MS	There are nine important metabolites in the flavonoid biosynthesis pathways (specifically in the coumarin, phenylpropanoid, and isoflavonoid pathways).	In-house database (550 metabolites)	[143]
Shaken black tea (SBT) - tea leaves of the "Fudingdabai" variety	Total 77 compounds 12 flavonoids	UHPLC-Q-TOF-MS	During SBT processing, the oxidation reaction of catechins (EGCG, EGC, EC, GCG, CG) occurs, and its content decreased significantly. Fermentation is the key process that causes the variations of these metabolites.	In-house database (Shanghai Biotree Biotech Co., Ltd) Public databases (Massbank and Metlin)	[123]
Leaves of *Cannabis sativa*	Total 79 compounds 16 phenolic acids 26 flavonoids	LC-ESI-Q-TOF-MS	In C. *sativa*, there are 7 flavonoid aglycones including orientin, vitexin, isovitexin, apigenin, luteolin, kaempferol, and quercetin, and cannflavin A is the unique metabolite.	MS-DIAL 4.8 CFM-ID 4.0 Agilent MassHunter	[144]

Table 13.
Some recent examples of the nontarget screening method used for chemical profiling of the plant extracts.

[143, 145]. By comparing the obtained MS-MS spectra with fragmentation patterns in these MS/MS libraries, the chemical structure of phenolics could be confirmed. To save time and work, some advanced software like XCalibur, MassLynx, Analyst, MassHunter, Chemstation, Compound Discoverer, Trace Finder, MZmine, or MetabolitePilot should be used to perform data and spectrum processing, as well as to conduct comparative analysis automatically [144, 146, 147]. Furthermore, the self-built or in-house standards database could be created by using PeakView™ and LibraryView™ (AB Sciex) [114, 148].

3.2.2.5 Applications

There are four main applications of nontarget screening methods for plant phenolics:

- Determining phenolic compounds in different parts of plants and extracts obtained from various extraction methods to evaluate and estimate their potential bioactivities as well as to optimize the extraction process [119, 120, 144, 148, 149].

- Providing phenolic profiles for metabolomics and establishing chromatographic fingerprints to differentiate cultivars, origins, and harvest times, enabling trace sources and grade quality of medicinal plants by combining MS methods with chemometric analysis [145, 147, 149–151].

- Providing information regarding structural changes of phenolic compounds during food processing [143, 152, 153].

- Detecting and identifying metabolites of phenolic compounds in both the in vitro and in vivo assays to characterize their bioavailability and bioaccessibility after consumption as well as to determine their metabolic processes [145, 146, 154–156].

4. Conclusion

In this chapter, the principles and advances in extraction techniques and analytical methods were reviewed. Some conventional extraction techniques like maceration, percolation, and Soxhlet are widely used as models to compare the efficiencies of alternative methods. The emergence of unconventional techniques including UAE, MAE, SFE, and PLE provides better extraction performance and recovery yields when extracting phenolic compounds.

In terms of analytical methods, the use of HPLC coupled with a PDA detector for determining phenolic compounds is prevalent because of its affordable cost, robustness, and reliability. Besides, chromatographic fingerprint analysis based on utilizing HPLC or UHPLC-PDA has been recognized as an innovative, rapid, and comprehensive method for the identification and qualification of phenolics. For qualification purposes, HRMS is a powerful technique, especially when coupled with LC. Recent advances in analytical methods facilitate the development of both targeted and nontargeted analysis, providing information about the chemical structure of unknown compounds and phenolic profiles of many plants.

The lack of analytical standards and reference mass spectrum databases, as well as structural diversity, complex matrices, and high costs, is a major challenge in the quantification and identification of phenolics using the LC-MS system. Therefore, more advances are expected in the coming years in relation to miniaturized, automated, portable, and fully integrated LC-MS; developing data processing software and a full-fledged database focused on phenolic acids and flavonoids; and the combination of MS and NMR spectrometry to overcome the drawbacks of MS in identifying phenolics stereochemistry.

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Chapter 3

Isolation and Identification of Phenolic Compounds

Maria Inês Rouxinol

Abstract

Isolation and identification of phenolic compounds are crucial processes in the field of natural product chemistry and biochemistry. Phenolic compounds are secondary metabolites widely distributed in plants, exhibiting diverse biological activities with potential health benefits. The isolation involves extracting these compounds from plant sources using various techniques such as solvent extraction, steam distillation, or solid-phase extraction. Following isolation, identification is accomplished through sophisticated analytical methods like high-performance liquid chromatography, gas chromatography-mass spectrometry, and nuclear magnetic resonance spectroscopy. These methods allow researchers to characterize and quantify specific phenolic compounds, elucidating their structures and understanding their roles in plant physiology and human health. The isolation and identification of phenolic compounds contribute significantly to the exploration of natural resources for pharmaceutical, nutritional, and industrial applications.

Keywords: phenolic compounds, natural products, analytical methods, secondary metabolites, plant extracts

1. Introduction

Polyphenols represent a broad group of compounds primarily occurring in plants as secondary metabolites. They are produced as a natural part of plant growth and in reaction to diverse biotic and abiotic stress. This class of compounds, which includes simple phenols, hydroxybenzoic acids, cinnamic acid derivatives, flavonoids, coumarins, stilbenes, tannins, and others, is derived from the amino acids phenylalanine and tyrosine [1]. The selection of appropriate methods for the extraction and quantification of phenolic compounds is extremely important to find the intricate chemical profiles present in complex matrices [2]. Beyond its influence on the precision and trustworthiness of analytical findings, it's also essential for a method to effectively capture the unique characteristics of specific matrices. Phenolic compounds are widely distributed in nature. They exhibit structural diversity and reactivity, making the choice of methods used to extract and quantify them critical to the success of any analytical endeavor. In order to gain meaningful insight into the complex world of phenolic compounds across different scientific disciplines, it is essential to tailor methods to the nature of the matrices under investigation and to align them with the

desired results. This chapter explores the central role of method selection in achieving precision and relevance in phenolic compound analysis.

Phenolics are commonly extracted using solvents, which could either be organic or inorganic. The yield of phenolics is influenced by various factors such as extraction duration, temperature, solvent-to-sample ratio, number of extractions, and solvent type. The achievement of optimal phenolic recovery varies across samples, relying on the plant type and its active compounds. Extraction solvents include water, acetone, ethyl acetate, and various alcohols (such as methanol, ethanol, and propanol), along with their combinations [2]. Due to all their properties, there is a growing demand for highly sensitive and selective analytical methods for the determination of polyphenols. The methodologies for phenolic compound quantification are based on the extraction and isolation of compounds. Extraction is a very important step, however there is no single and standard extraction method. In typical procedures, it's essential to disrupt the samples, whether through grinding, drying, or lyophilizing, before conducting solvent extraction. In addition, with the realization of these methodologies, non-phenolic compounds such as sugars, organic acids, and proteins are also extracted, which may require requiring subsequent purification processes (for example, solid-phase extraction). At analytical level, the extraction method has a great influence on the phenolic amount and composition [3]. The selection of an appropriate extraction method is crucial for the recovery of phenolic compounds from plant samples, as single-step extraction and unsuitable methods can impact the overall recovery rate [4]. Determining and quantifying phenolic compounds can present challenges due to their intricate nature and structural diversity. Consequently, numerous methods are recognized and employed for quantifying phenolic compounds in plant extracts [5].

2. Methods for phenolic compounds extraction

2.1 Conventional extraction

Maceration, a widely used traditional extraction method for phenolic compounds, relies on the principle of "like dissolves like." Typically employing ethanol or methanol as solvents, this technique extracts organic phenolic compounds from plant samples [4]. Accelerating the process often involves using a shaking incubator for enhanced contact between samples and solvent, with temperature control. While higher temperatures increase solubility and diffusion, it's crucial to avoid overheating to prevent solvent loss and phenolic compound decomposition [4, 6]. The effectiveness of conventional extraction is influenced by the duration within a specific time range. Higher extraction durations generally lead to increased efficiency until a solute equilibrium is reached between the inside and outside of the solid material. To achieve a higher extraction yield, a greater solvent-to-solid ratio is necessary. However, an excessively high ratio results in the use of excessive extraction solvent, demanding a longer extraction time. Balancing these factors is crucial for optimizing extraction efficiency [4].

2.2 Ultrasonic-assisted extraction (UAE)

Ultrasound is employed in extraction processes to reduce extraction time and enhance quality by inducing solvent-producing cavitation and high shear forces.

Ultrasound-assisted extraction (UAE) is a modern method known for its simplicity, energy efficiency, and high reproducibility, offering a substantial yield of active compounds [7]. UAE is more efficient, requiring less solvent and a shorter extraction duration compared to conventional methods [4]. Mainly utilized in solid/liquid systems, UAE disrupts the cellular walls of plant materials, facilitating mass transfer across membranes and increasing solvent access to analytes. Extraction efficiency in UAE is influenced by factors like solvent composition, solvent-to-sample ratio, ultrasound amplitude and cycle, solvent pH, and temperature [8, 9]. While stronger ultrasonic applications can accelerate changes, cost considerations in food industries often lead to optimized applications for the best results with minimal energy usage [9]. To enhance extraction efficiency, factors such as amplifying ultrasound power, minimizing moisture content in food materials, and controlling temperature are considered. Proper selection of ultrasound frequency is essential, impacting the size of bubbles produced during resonance [4]. Solvent selection and temperature were identified as crucial factors impacting UAE efficiency [8, 9]. For highly polar phenolic compounds, extraction with pure organic solvents may exhibit low efficiency, making ethanol/methanol mixtures with water in various proportions commonly used as effective extraction solvents [9].

2.3 Reflux extraction

Reflux extraction, also known as solvent recycling reflux extraction, simultaneously extracts and concentrates the solvent [10]. The process involves two main components: the extraction tank and the concentration tank. The solvent is pumped from the extraction tank to the concentration tank during extraction, where it is heated, evaporated, condensed, and returned to the extraction tank. This cycle is repeated to accumulate extracts. Reflux extraction offers advantages such as shorter extraction time, reduced solvent cost, and lower fixed investment compared to conventional extraction. However, it has drawbacks, including potential contamination or decomposition of phenolic compounds during the concentration and heating stages. While reflux extraction has its advantages, it cannot fully replace conventional extraction [4].

2.4 Microwave-assisted extraction (MAE)

In recent years, there has been a growing emphasis on reducing the use of organic solvents in extractions, leading to the development and optimization of microwave-assisted extraction (MAE). Microwaves, characterized by electric and magnetic fields oscillating perpendicularly in a high-frequency range (0.3–300 GHz), induce localized heating. As a consequence, the plant matrix is destroyed, thereby facilitating the smoother diffusion of the desired compounds into the solvent [1, 11]. Usually, microwave powers ranging from 300 to 900 W and extraction temperatures spanning from 50 to 100°C are utilized in MAE [9]. In comparison with conventional extraction, MAE facilitates selective migration of target compounds within a shorter timeframe due to highly localized temperature and pressure. MAE achieves similar or higher recoveries than conventional extraction while requiring less space, time, and solvent [4, 12, 13]. In MAE, product recovery is boosted by the heating effect of microwaves, akin to conventional extraction methods. Although microwave heating is faster than conventional heating, it can lead to increased energy expenses. When comparing MAE to UAE and conventional methods, it's crucial to consider the precise

energy costs, particularly factoring in electricity expenses [8]. However, the potential for super boiling during MAE must be considered, especially when the penetration depth characteristic for the solvent is larger than the sample size. Scaling up MAE from laboratory to industrial levels requires careful consideration of solvent and sample sizes to avoid false results and safety issues [13]. The primary challenge associated with this extraction technique is achieving maximum extraction yield by effectively breaking down cellular tissue without compromising the chemical structure of the natural compounds. This balance is crucial for obtaining high-quality extracts through MAE [1, 9, 11, 14].

2.5 Soxhlet extraction

Soxhlet extraction stands out among conventional methods for phenolic compound extraction, requiring less solvent and time, resulting in low processing costs. The extraction device is user-friendly and suitable for initial and bulk extraction with a good recovery rate [15]. This methodology is an enhanced method derived from reflux extraction that integrates the advantages of percolation by enabling continuous extraction through reflux and siphon mechanisms. Although it offers automation and requires less solvent and time compared to conventional extraction methods, Soxhlet extraction's reliance on thermal processes may lead to thermal degradation with prolonged heating [4]. This method has the potential to yield higher total phenolic and tannin content than conventional extraction. However, it's crucial to note that with more stages, the high temperature involved in Soxhlet extraction may lead to the decomposition of phenolic compounds, as shown in an example by Ouahida et al. [16].

2.6 Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) (also known as accelerated solvent extraction—ASE) involves placing solid samples into a robust container saturated with extraction solvents, followed by a 5–15 minutes extraction period conducted at elevated temperatures and pressures [8]. Increased pressure enables solvents to stay in a liquid state above their boiling points, boosting the solubility and diffusion rate of lipid solutes and aiding solvent penetration into the matrix. PLE, when contrasted with conventional extraction methods, diminishes solvent and time demands while showcasing improved repeatability [4]. Widely employed by scientists for extracting natural products such as anthocyanin and saponins, PLE requires the same or even lower volumes of solvents compared to conventional techniques. It is a time-saving method that minimizes sample handling [6, 17, 18]. However, the mechanism of PLE, allowing solvents to remain in liquid form at high pressure and temperature, can lead to heat degradation. To enhance efficiency, Ju and Howard [17] recommend combining PLE with less efficient solvents at low temperatures, like water [17]. By adjusting process parameters, PLE enables faster extraction and enhanced selectivity for specific compound groups. The use of high pressure guarantees that solvents maintain their liquid form even when temperatures are raised, thereby enabling efficient extraction at elevated temperatures. Such conditions enhance the solubility of desired compounds and promote faster desorption from plant matrices. Furthermore, because PLE is carried out within a sealed system, the likelihood of oxidation reactions occurring is minimized [9]. Studies indicate that ASE efficiency is optimized when solvent mixtures, such as methanol or ethanol in water, are used instead of pure

solvents, considering polarity compatibility [9]. Working pressures within the range of 4–20 MPa impact solvent diffusion into the matrix pores, promoting better contact between target compounds and the solvent [11, 19]. Temperature is a crucial parameter, and research suggests that an increase in phenolic extraction efficiency occurs within the temperature range of 40–120°C [11].

2.7 Supercritical fluid extraction (SFE)

Supercritical fluid extraction (SFE) is an eco-friendly technology utilizing supercritical fluids (SFs), characterized by critical values of pressure and temperature, to extract bioactive components from vegetal materials [11, 20]. It uses SF, such as supercritical carbon dioxide (S-CO_2), as a solvent for extraction. SF exhibits properties similar to both liquids and gases, making it ideal for dissolving a wide range of natural materials. S-CO_2, a commonly used SF, offers advantages such as low critical temperature, selectivity, inertness, and non-toxicity, making it suitable for extracting non-polar materials like lipids. However, for extracting phenolic compounds, co-solvents are often needed to enhance solubility [4]. SFE is recognized for producing clean extracts, avoiding oxidation, and degradation of phenolic compounds that can occur with conventional extraction methods. It is also acknowledged as safe by regulatory bodies like the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) of the United States [11, 21]. However, a limitation of this approach is that SC-CO_2, being a non-polar solvent with an affinity for non-polar or low-polar compounds, exhibits low solubility for polyphenols, resulting in low extraction yields [20]. To overcome this constraint, research has investigated incorporating chemical modifiers or co-solvents, including water, methanol, ethanol, acetone, acetonitrile, or a mixture of acidified ethanol and water, to alter the non-polar characteristics of supercritical CO_2 [21]. Despite its efficacy, the high cost limits the widespread use of SFE, making it primarily applicable to high-value products [22, 23].

2.8 Pulsed electric field extraction (PEF)

Pulsed electric field (PEF) is a non-thermal extraction method that employs short, high-voltage pulses to disrupt membrane structures, enhancing extraction yield by releasing cellular content. Efficiency in PEF is influenced by factors such as field strength, specific energy input, pulse number, and temperature. The intact cytomembrane in plant cells acts as a semipermeable barrier, controlling substance movement in and out. PEF treatment disintegrates the cell membrane, increasing cell wall permeability, and allowing more bioactive compounds to be released into solvents. This structural disintegration destroys selective permeability, facilitating the extraction of more substances. PEF does not require heating, minimizing heat generation, and preventing the degradation of thermolabile compounds [4, 24].

2.9 Enzyme-assisted extraction (EAE)

Enzyme-assisted extraction (EAE) involves using enzymes to hydrolyze cell membrane components, disrupting their selective permeability and enhancing the extraction rate by releasing compounds [8]. Enzymatic hydrolysis, a commonly employed and safe extraction method in numerous food applications, utilizes the enzymatic action of cellulases, pectinases, and hemicellulases to break down cell

walls, thereby improving the extraction of valuable compounds from plants [25]. Additionally, the enzymatic activity of lyases and hydrolases on glycosidic fractions of natural polyphenols improves their biological properties, enhancing bioactivity and bioavailability [26]. The cell membrane structure, primarily composed of macromolecules like polysaccharides and proteins, is susceptible to denaturation under high temperatures, affecting extraction efficiency. Enzymes, such as cellulase, are applied in EAE as a nonthermal and nontoxic treatment to boost efficiency [4]. Mixtures of enzymes, including pectinases, endo- and exo-glucanases, β-glucosidases, β-galactosidase, and cellobiases, are employed to achieve an overall synergistic effect [27, 28]. Cellulose and peptinase in enzymatic hydrolysis are utilized to release polymeric polyphenols, theoretically considered "non-extractable," from the plant matrix [28]. However, EAE is more complex compared to chemical- and physical-assisted extraction methods. To achieve high-quality extracts, a detailed understanding of sample composition and the suitable enzyme for extraction is necessary. Additionally, enzyme activity is influenced by factors like pH, temperature, and substrate concentration, adding to the intricacy of EAE [4]. While an increase in temperature enhances mass transfer rates and solubility, temperatures below 60°C are typically used to avoid enzymatic denaturation. An environment with pH values ranging between 4.0 and 6.5 is optimal for enzymatic system activity [25, 27].

2.10 Extraction with ionic liquids

Recent research has delved into extracting bioactive constituents from herbal medicines or other plant-based sources using ionic liquids (ILs) and deep eutectic solvents (DESs) as alternative solvents. These studies showcase their potential to supplant traditional organic solvents [29]. ILs, a category of organic salts, exist in a liquid state below 100°C and comprise an organic cation and an inorganic or organic anion [8]. The choice of solvent polarity, hydrophobicity, viscosity, and miscibility can be customized by selecting either the cationic or anionic component. Various combinations of cations and anions lead to a broad spectrum of physicochemical properties, influenced notably by the nature and size of the cation, especially the anion. Depending on their composition, resulting ILs may display hydrophobic or hydrophilic traits, varying viscosity, compatibility with water or other organic phases, and distinct electrochemical characteristics [6].

3. Chromatographic techniques for separation, identification, and quantification

The quantification, purification, separation, and identification of specific phenolic compounds (including anthocyanins) are dependent on expensive equipment with lengthy sample preparation. To identify and quantify anthocyanins, methods such as paper chromatography (PC), thin-layer chromatography (TLC), column chromatography, solid-phase extraction, counter-current chromatography, and high-performance liquid chromatography (HPLC) are used [30–33]. Both gas chromatography (GC) and HPLC coupled with mass spectrometry (MS) have proven utility in detecting phenolic compounds in various samples [34, 35]. These methods are accurate and efficient, providing reliable results within short analysis times. As HPLC techniques, simultaneous identification of phenolics with a wide range of polarities becomes increasingly possible [34, 35]. Despite HPLC and GC being the

most used techniques, they are expensive and require specific equipment, other chromatographic techniques are still used to determine phenolic compounds.

3.1 Paper chromatography (PC) and thin-layer chromatography (TLC)

Paper chromatography (PC) and thin-layer chromatography (TLC) are partitioning techniques utilized for the separation of phenolics in foods [36]. While PC is a simpler and less commonly used method compared to HPLC and GC, it has proven effective for separating and identifying phenolic compounds in tea leaves and green leafy vegetables [37, 38].

TLC emerges as an easy and reliable technique, especially for analyzing phenolics in crude plant extracts. Various TLC techniques are cost-effective and allow for multiple detections on the same TLC plate within a short analysis time. Silica gel TLC-based video imaging has been identified as a valuable complementary fingerprint technique for identifying phenolic acids and flavonoid fractions from different sage species [39, 40].

3.2 High-speed counter-current chromatography (HSCCC)

High-speed counter-current chromatography (HSCCC) is a biphasic liquid-liquid partitioning method widely employed for the isolation and separation of various natural compounds [36, 41–43]. Operating without a solid support, HSCCC facilitates the permanent adsorption of sample compounds, allowing for the isolation and purification of natural compounds from crude extracts without preparation [44].

3.3 Capillary electrophoresis (CE)

Capillary electrophoresis (CE) is a high-resolution technique utilizing a narrow capillary column with a solution of ions. CE is suitable for rapidly and efficiently identifying charged low- and medium-molecular-weight compounds, with low sample and reagent volume requirements [45]. Although there is a scarcity of research on utilizing CE for the separation and identification of phenolics in plant materials [46–49], micellar electrokinetic chromatography, capillary electrochromatography, and capillary zone electrophoresis coupled with various detection methods are widely employed in CE separation [6, 50].

3.4 Supercritical fluid chromatography (SFC)

Supercritical fluid chromatography (SFC) stands out as a versatile technique for analyzing and identifying phenolics, offering high separation efficiency, resolution, short analysis time, environmental friendliness, and compatibility with different detectors [51, 52].

3.5 Gas chromatography (GC)

Gas chromatography stands as another exceptionally efficient method for isolating, identifying, and quantifying various phenolic species. The primary limitation in GC analysis is the low volatility of phenolic compounds, necessitating their derivatization [53, 54]. Numerous analytical techniques for identifying and quantifying phenolic compounds rely on gas chromatography-mass spectrometry (GC-MS) [55].

Nevertheless, the majority of methods concentrate on a limited number of specific compounds. There is generally a lack of an efficient approach for identifying and quantifying a broad spectrum of trace phenolic compounds in wastewater. The recent development of techniques such as retention time locking (RTL) and deconvolution report software (DRS) addresses this issue by enabling multi-residue analysis. These methods facilitate the simultaneous identification of numerous target compounds, even in cases where they may be obscured by co-eluting matrix compounds. Hence, it is recommended to develop a screening method for identifying target phenolic compounds from a considerable pool of candidates utilizing DRS and RTL. Despite DRS's ability to qualify and quantify compounds listed in libraries, there is presently no dedicated library specifically designed for phenolic compounds [56].

3.6 High-performance liquid chromatography (HPLC)

The quantification and identification of phenolic compounds have been widely studied using high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD) [57–62]. In the analysis of complex matrixes, chromatographic techniques, specifically HPLC, are deemed more suitable due to their sensitivity, allowing the separation and identification of various anthocyanins in complex matrixes, and providing specific information. Although they are recommended, the multitude of protocols available can pose challenges in selecting the optimal method for determining phenolic compounds or anthocyanins. However, the use of HPLC for flavonoid determination enables the identification of different compounds in the samples, offering insights into the individual flavonoid profiles of various grape varieties. Nonetheless, the identification of each compound is challenging due to the limited availability of commercially accessible standards [58, 63, 64].

Currently, the most used methods for analyzing phenolic compounds involve HPLC coupled with ultraviolet detection, electrochemical detection, MS, or particle beam/electron ionization mass spectrometry. Moreover, GC coupled with MS, HSCCC, chiral CE, or Fourier transform near-infrared reflectance spectroscopy are frequently employed techniques. Hyphenated approaches, such as HPLC-MS and HPLC-MS/MS, which are built on HPLC separation, offer insights into the molecular mass and structural characteristics of compounds. These methods are deemed more advantageous than alternatives in terms of their efficiency, suitability for routine analysis, and effectiveness in the separation, identification, and quantification of phenolic content [65]. Reversed-phase HPLC is frequently employed for the analysis of various phenolic groups. Ultra-performance liquid chromatography has been used to enhance the analysis of phenolic compounds in diverse matrices [66].

3.6.1 Selective columns and stationary phases

Choosing appropriate columns and stationary phases is essential to ensure precise and selective determination of phenolic compounds in chromatographic techniques. It's crucial to take into account the physicochemical properties of the compounds, the intended separation mechanism, and the specific analysis requirements when making these selections. Experimentation and method optimization may be required to achieve the best results for a particular set of phenolic compounds. A summary of the different columns and stationary phases that can be used to determine phenolic compounds can be found in **Table 1**.

Chromatography	Stationary phase	Selectivity	Applications	Refs.
Reverse-phase chromatography	C18 (Octadecylsilane)	Separation based on the hydrophobicity	Wide range of phenolic compounds	[60, 67]
Normal-phase chromatography	Silica gel or other polar materials	Separation based on their polarity	Useful for less polar phenolic compounds	[68, 69]
Ion-exchange chromatography	Positively or negatively charged resins	Separates based on ionic interactions	Effective for charged phenolic compounds	[70, 71]
Size-exclusion chromatography	Porous gels with different pore sizes	Separates based on molecular size	Useful for polymers and large phenolic compounds	[72, 73]
Hydrophilic interaction chromatography	Polar materials, such as silica with bonded polar functional groups	Separates based on both hydrophilic interactions	Suitable for polar phenolic compounds	[74, 75]
Chiral chromatography	Chiral selectors	Resolves enantiomers of chiral phenolic compounds	Necessary when stereoisomer separation is critical	[76, 77]
Mixed-mode chromatography	Combines different separation mechanisms	Offers versatility in separation	Useful for complex samples containing various phenolic compounds	[78, 79]

Table 1.
Different stationary phases are used to separate and identify phenolic compounds.

4. Spectroscopy in phenolic compounds quantification

Colorimetric techniques are extensively employed in UV/Vis spectrophotometry due to their simplicity, speed, suitability for routine laboratory use, and cost-effectiveness. Despite the numerous advantages of UV/Vis-based colorimetric methods, they require the use of reference substances (such as gallic acid) to ensure accurate quantification of the phenolic hydroxyl groups within samples [5]. When in need of determining the total phenolic content or anthocyanins, spectrophotometric methods like Folin-Denis, Folin Ciocalteu, or differential pH are widely used. However, these methods are widely used but since they are not specific for matrixes like grape juice, they tend to overestimate the content due to the lack of selectivity. For matrices such as grape juices or extracts, quantification and identification should be conducted using more specific methods, such as chromatographic techniques. Although these methods are more recommendable, the diversity of protocols found can difficult the choice to select the best to determine phenolic compounds [80].

Polyphenols within plant extracts interact with redox reagents such as the Folin-Ciocalteu reagent, forming a blue complex that can be measured using visible-light spectrophotometry. The Folin-Ciocalteu reaction relies on creating a blue chromophore comprised of a phosphotungstic-phosphomolybdenum complex, with the maximum absorption of chromophores contingent upon the alkaline solution and the concentration of phenolic compounds within the plant extract. Due to the rapid

degradation of the reaction in alkaline environments, lithium salts are incorporated into the Folin-Ciocalteu reagent to prevent turbidity and facilitate analysis [5].

There are also assays that are used for specific groups of flavonoids. Examples include aluminum complexation assays and the spectrophotometric method using 4-dimethylaminocinnamaldehyde. For instance, flavonols and flavones can produce yellow complexes with Al(III) under neutral pH conditions, with the solution's absorbance measured in the 400–430 nm range. Furthermore, Al(III) can generate red complexes with specific flavonoids (such as rutin, luteolin, and catechins) when sodium nitrite is present in an alkaline environment. The absorbance of the resulting solution is subsequently measured at 510 nm [81, 82].

Conventional techniques like the Folin-Ciocalteu method and aluminum chloride complexation are employed for the assessment of total phenolic and flavonoid contents post-extraction [83]. However, the Folin-Ciocalteu reagent has a tendency to react with other non-phenolic reducing substances, leading to an overestimation of the total phenolic content [66].

5. Nuclear magnetic resonance in phenolic compounds identification

High-resolution 1H-NMR spectroscopy has recently demonstrated utility in analyzing complex mixtures without the need for prior separation of individual components within the mixture [84]. Specifically, 1H-NMR spectroscopy was employed for the quality control and authentication of olive oil [85]. The utility of 1H-NMR spectroscopy is increasingly acknowledged for its non-invasive nature, rapidity, and sensitivity to a broad array of compounds in a single measurement, obviating the necessity for sample pre-treatment [86]. At room temperature, the 1H-NMR resonances of phenolic −OH groups exhibit broad signals primarily because of intermolecular exchange between the −OH protons and protons from protic solvents or residual H_2O in aprotic solvents. Additionally, further exchange broadening may occur due to proton exchange among different −OH groups and between −OH and −COOH groups, particularly in low-polarity and low dielectric constant organic solvents, as a result of intermolecular association of solute molecules. The linewidths of phenol OH signals (the spectral width or breadth of the signals corresponding to the hydroxyl (OH) group) are crucial for the assignment and interpretation of 1H-NMR spectra [87].

6. Conclusions

In conclusion, the extraction and identification of phenolic compounds from plant sources involve a diverse array of methodologies, each offering unique advantages and applications. Traditional methods, such as maceration and reflux extraction, provide valuable insights into the extraction efficiency influenced by factors like time, solvent ratio, and temperature. The incorporation of UAE and MAE accelerates the process while maintaining extraction quality. Chromatographic techniques offer robust options for separating and identifying phenolics. In the realm of chromatography, GC and HPLC coupled with MS remain indispensable, providing accuracy and efficiency in phenolic compound detection across diverse samples. This comprehensive overview underscores the importance of a tailored approach in selecting extraction and identification methods based on the specific characteristics of the target phenolic

compounds and the nature of the plant matrix. The synergistic application of these diverse techniques contributes to a deeper understanding of phenolic composition in plant materials and supports advancements in the fields of food science, pharmacology, and natural product research. The inclusion of more objective composite analyses as part of routine procedures will result in the classification of more reliable and consistent data and ensure the quality.

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Conflict of interest

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Chapter 4

Polyphenols from Winery by-products: Conventional *versus* Unconventional Extraction Methodologies

Rui Dias-Costa, Marta Coelho, Raúl Domínguez-Perles, Irene Gouvinhas and Ana Novo Barros

Abstract

A large number of studies have already demonstrated that winery by-products (WBPs) are a valuable source of natural antioxidants, especially due to their phenolic content. These residues can be reused as new ingredients in the food, cosmetic, and pharmaceutical industries. For that reason, a scientific foundation for the comprehension of extraction methods' efficiency is essential for starting the reuse of these by-products on a large scale. Numerous phenolic compounds extraction techniques under different conditions are currently being investigated. There has been a growing scientific interest in these phytochemicals, driven by the adoption of more eco-friendly extraction techniques that facilitate higher extraction yields. To extract the phenolic compounds present in WBPs, conventional methods as well as nonconventional extraction methods can be employed. The first ones, which have been used for a very long period, include Soxhlet extraction, maceration, reflux extraction, and others. Nonconventional methods, widely recognized as eco-friendly methods, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and supercritical extraction (SE), among others, provide higher extraction yields and high-quality extracts. This chapter will explore the extraction methodologies of phenolic compounds from WBPs produced by the wine industry, with a focus on both conventional and unconventional techniques. Additionally, the grape varieties mentioned in this review are suitable for production in Portugal under Designation of Origin (DO) and Geographical Indication (GI) classifications.

Keywords: winery by-products, phenolic compounds, extraction techniques, conventional methods, unconventional methods

1. Introduction

In the field of wine production, a significant amount of solid organic and inorganic materials is generated, none of which form part of the final wine product.

These materials originated as a result of a vitivinicultural process and can be classified into wastes, residues, winery or wine sub-products, and winery or wine by-products [1]. WBPs include a diverse range of materials resulting from wine processing, such as wastewater sludge, grape stems, grape pomace (seeds, skins, and pulps), wine lees, and vine pruning woods [2–7]. Approximately 30% of the total volume of vinified grapes amounts to WBPs, totaling nearly 20 million tons, with 50% of this amount attributed to the European Union [8]. These by-products can be recycled, reused, or recovered, with a view to a circular economy approach, with an improvement in the economic and environmental sustainability of the wine industry [9].

The extraction of phenolic compounds from plant sources is essential for unlocking their numerous potential benefits, as the quantity and composition of these compounds depend on the extraction methods used. It is necessary to optimize extraction methods by balancing yield, selectivity, and sustainability.

The extraction yield of phenolic compounds can be influenced by various factors, including their chemical structure, the number and position of hydroxyl groups, and molecular size. Additionally, parameters such as temperature, solvent type and composition, contact time, particle size, and interactions with other food ingredients also play a crucial role [10–13].

Traditionally, extraction methods heavily relied on organic solvents, raising concerns regarding their impact and health risks due to their toxicity and flammability. Furthermore, the necessity of boiling in these methods contributes to the loss of valuable products, such as polyphenols [2, 14, 15]. However, there has been a significant change toward nonconventional extraction techniques, driven by the increasing demand for sustainable and environmentally friendly practices. These innovative approaches aim to minimize solvent usage, energy consumption, and overall environmental footprint. In fact, there has been a growing scientific interest in these compounds, driven by the adoption of more eco-friendly extraction techniques that facilitate higher extraction yields [16]. On the other hand, conventional extraction methods have certain drawbacks that need to be addressed. To overcome these challenges, unconventional extraction methods have been developed to bridge the gaps left by traditional approaches [17].

In this chapter, the WBPs generated by the wine industry will be described, along with the conventional and nonconventional methods used for the extraction of phenolic compounds from these by-products, which originate from grape varieties suitable for production in Portugal under Designation of Origin (DO) and Geographical Indication (GI) classifications [18]. Additionally, our aim is to provide an overview of published research to simplify and optimize phenolic extraction procedures, achieving higher extract yields and purities.

2. Winery by-products

Winery by-products (WBPs), such as grape stems, pomace, wine lees, and vine pruning wood (**Figure 1**), are produced by winemaking companies and contain valuable bioactive compounds that are often overlooked yet hold significant potential for applications across various industries [2]. They have been recognized as a natural source of polyphenols, namely hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, flavonols, flavan-3-ols, flavones, flavanones, flavanonols, proanthocyanidins, anthocyanins, among others.

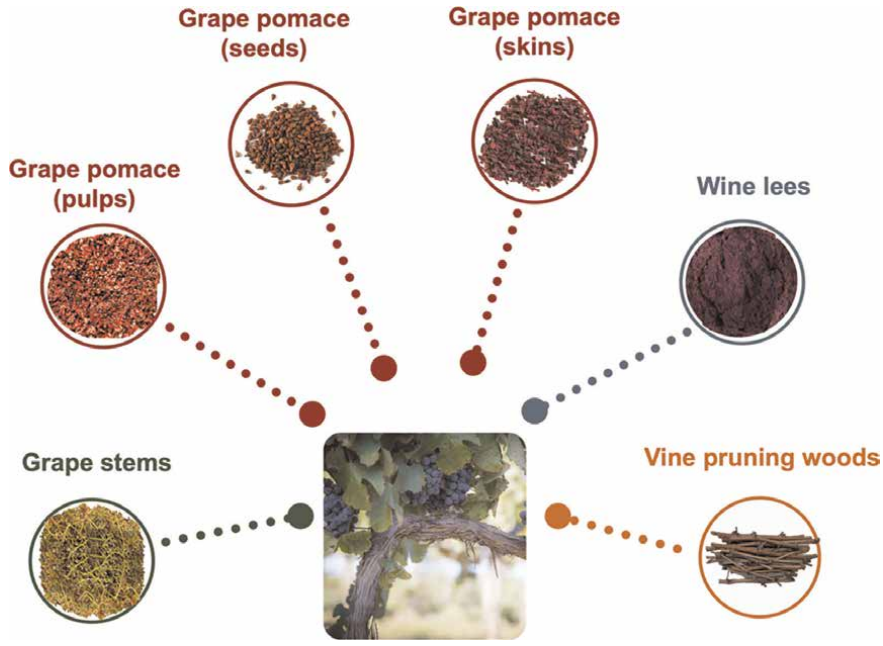

Figure 1.
Illustrative image of winery by-products.

2.1 Grape stems

Grape stems, also known as grape stalks, represent a significant portion of wine by-products, with approximately 30 kg being produced for every 1000 kg of grapes harvested during the destemming phase [19]. To prevent excessive astringency and negative effects on the wine's organoleptic characteristics, grape stems are typically removed before the vinification stages. This practice, particularly the removal of stems prior to maceration in red winemaking, is commonly associated with improved wine quality [5, 20]. This potential material constitutes approximately 25% of the total by-products generated by the wine industry [6, 20–23]. Their composition comprise approximately 17–26% lignin, 20–30% cellulose, 3–20% hemicelluloses, and 6–9% ash [21]. This byproduct is commonly used in landfilling, landfarming, or composting [24]. However, it has the potential to be utilized as a source of bioenergy in the form of pellets or through chemical and biological processing, yielding valuable food additives, materials, or chemicals [25].

2.2 Grape pomace

Grape pomace, also referred to as grape marc, is the main solid residue generated by the wine industry, resulting from the pressing of whole grapes during must production. It accounts for approximately 20–25% of the total weight of the original grapes and is primarily composed of seeds, skins, pulp, and residual stems [6, 26, 27]. One ton of this material is constituted by 425 kg of grape skin, 225 kg of grape seeds, 249 kg of grape stems, and other minor constituents [28].

The whole grape pomace is produced during the winemaking process, following fermentation for red grapes and preceding it for white grapes [6, 26]. It includes a diverse array of elements, including structural carbohydrates, lignin, oil, polyphenols, unfermented sugars, pigments, and alcohol [29–34]. Notably, when considering its nutritional value, polyphenols stand out as the primary constituents of grape pomace [30–34]. Traditionally, this winery byproduct (WBP) has been harnessed for the production of numerous products such as distillates, fertilizers, and animal feed [6]. Moreover, it can serve as a valuable source of tartaric acid, malic acid, citric acid, ethanol, dietary fiber, grape seed oil, and alcoholic beverages through a concise process of short fermentation and distillation [2, 5].

2.3 Wine lees

In accordance with European Commission (EC) Regulation No. 337/79, wine lees, also referred to as dregs, are defined as "the solid residue that precipitates at the bottom of wine containers following fermentation, during storage, or as a result of authorized treatments." This definition also encompasses the residual matter obtained after filtration or wine centrifugation processes [35]. Wine lees typically account for 2–6% of the total wine volume [6, 21] and are mainly made up of yeast cells, tartaric acid, inorganic matter, phenolic compounds, and ethanol [6, 36]. It can be categorized into three groups based on the vinification stage: alcoholic fermentation lees, malolactic fermentation lees, and lees that develop during the aging of the wine. Additionally, classification by particle size distinguishes between heavy lees and light lees [6, 37]. Traditional applications for wine lees include incineration, landfill disposal, land-spreading, distillation, tartaric acid production, utilization as coloring agents, and incorporation into nutritional supplements [21].

2.4 Vine pruning woods

The distinction between grapevine shoots, grapevine canes, and vine pruning woods is often unclear, as various authors frequently use different names to refer to the same byproduct. The terms "canes" and "shoots" are often used interchangeably [7]. In the literature, some authors used grapevine shoots [13, 38–43], others grapevine canes [44–48], and others vine pruning residue [49, 50]. Through the analysis of the articles and the sampling collection dates, the term remains the same because many samples are collected between November and March, the typical time for pruning operations. Noviello et al. [51] also mentioned that grapevine shoots are alternatively known as grapevine canes.

The agronomic practice of pruning results in the generation of a substantial volume of agricultural waste, primarily consisting of vine pruning wood. The annual estimated production of these WBPs is approximately 1–2 tons per hectare [6]. They are considered some of the most prevalent winery wastes [51]. Recent studies recognize them as a valuable resource rather than mere waste [41]. Their composition includes 34% cellulose, 19% hemicellulose, and 27% lignin [21]. This byproduct has diverse applications, including its use as biofuel, aggregate material, cellulose source, and in the production of activated carbon for wine treatment and pulp and paper manufacturing. Moreover, it serves as an alternative to oak chips as an oenological coadjuvant, enhancing the sensory profile of wines [42, 51]. Additionally, it is utilized in the production of ethanol, lactic acid, methanol, various fuels, biomass, and

biosurfactants. It also serves as a substrate in mushroom cultivation and is used for extracting volatile compounds [52, 53].

3. Extraction methodologies

Phenolic compounds from WBPs can be extracted using different methodologies. This section provides a review of the conventional and nonconventional extraction methods of these phytochemicals applied to these by-products of white and red grape (*Vitis vinifera* L.) varieties authorized for wine production in Portugal (**Figure 2**).

3.1 Conventional phenolic compounds extraction methodologies

Conventional solid-liquid extraction (SLE), also known as conventional methods, refers to extraction processes characterized by straightforward maceration in a solvent, either with or without stirring. These processes typically occur at atmospheric pressure and are conducted within a temperature range spanning from room temperature to the boiling point of the solvent under reflux conditions. These methods also encompass Soxhlet extraction, liquid-liquid extraction (LLE), and maceration [17, 48, 54]. Many factors significantly influence the outcomes of conventional extraction methods. These include the selection of extraction solvents, their polarity, the solvent-to-solid ratio, the number and positioning of hydroxyl groups, molecular size, extraction temperature and time, particle size, interactions with other food components, pH, and the influence of light [12, 48].

Phytochemicals are primarily extracted using conventional organic solvents such as methanol, ethyl acetate, and acetone, which exhibit outstanding extraction capabilities. However, their inherent health hazards, environmental impact, and concerns regarding the most suitable solvent pose significant challenges [12, 17, 36]. In contrast, ethanol, water, and their mixtures emerge as optimal extraction solvents due to their eco-friendly nature, allowing for their direct application in the food and pharmaceutical industries. These green solvents offer a safer and more sustainable choice

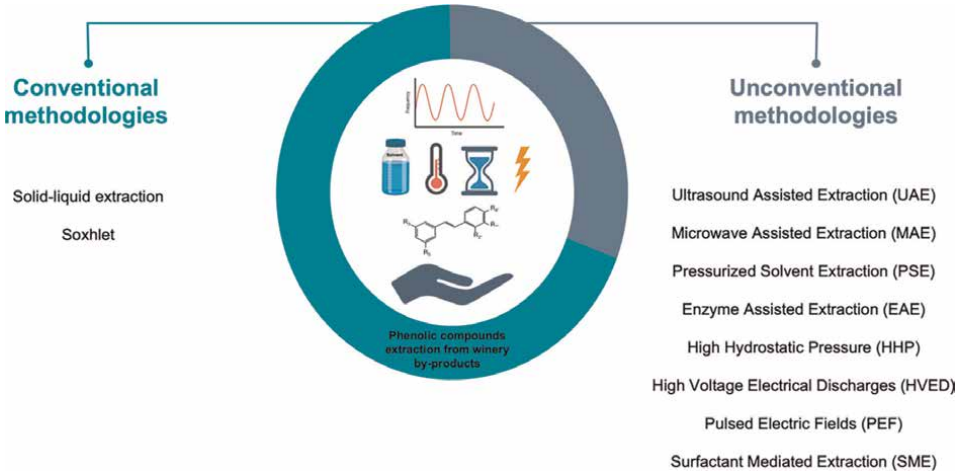

Figure 2.
Extraction methodologies of phenolic compounds from WBPs of grape varieties authorized for wine production in Portugal.

[12, 36]. However, there is no solvent that is generally accepted as the best for extracting polyphenols, according to the literature. However, it is generally accepted that solvents with a greater polarity tend to extract phenolics more effectively due to the high solubility of phenolics in these solvents [17].

Extensive research in the literature focuses on the extraction of phenolic compounds from WBPs. **Tables 1** and **2** present studies that have employed conventional extraction methods to extract phenolic compounds from WBPs of white and red grape

White grape varieties	Winery byproducts	Extraction conditions	References
Códega-do-Larinho	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
		SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Viosinho	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
		SLE with water, ethanol, ethanol:water (50:50, v/v) Temperature: 45°C Time: 1 h	[57]
		SLE with ethanol:water Solvent ratio: 1:125 (w/v) RSM Times analyzed: 10–30 min Temperatures analyzed: 25–95°C Solvents concentration: 5–90% Solvent used: food-quality ethanol	[58]
		SLE with methanol: water	[59]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[60]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Malvasia-Fina	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[62]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[60]
		SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]

White grape varieties	Winery byproducts	Extraction conditions	References
Moscatel-Galego-Branco	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[62]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[63]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[60]
	Grape pomace (whole pomace)	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Grape pomace (seeds)	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Síria	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Gouveio-Real	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
Arinto	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[55]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Borrado-das-Moscas	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
	Vine pruning woods	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
Encruzado	Vine pruning woods	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
Fernão-Pires	Grape stems	SLE with methanol:water	[59]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[60]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]

White grape varieties	Winery byproducts	Extraction conditions	References
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Rabigato	Grape stems	SLE with methanol:water	[59]
		SLE with methanol:distilled water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[60]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Verdelho	Grape stems	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Folgasão	Grape stems	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Grape pomace (whole pomace)	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Esgana-Cão	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Malvasia-Rei	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]

SLE, Solid-Liquid Extraction; RSM, Response Surface Methodology.

Table 1.
Studies performed on the extraction of phenolic compounds from WBPs of white grapes (Vitis vinifera L.*) varieties using conventional methodologies.*

varieties, respectively, considering solvent ratios, temperatures, extraction times, and other variables.

3.2 Unconventional phenolic compounds extraction methodologies

Nowadays, conventional extraction methods are increasingly being replaced by alternative extraction methods. These alternatives commonly use an energy source to enhance the transfer of phenolic compounds into the solvent, thereby providing numerous advantages over the conventional ones [12]. They are renowned for their efficiency, eco-friendliness, reduction in the amount of sample, and decreased energy and time consumption, in stark contrast to the conventional extraction methods, which typically involve higher solvent volumes, often accompanied by increased

Red grape varieties	Winery byproducts	Extraction conditions	References
Touriga-Nacional	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[66]
		SLE with water, ethanol, ethanol:water (50:50, v/v) Temperature: 45°C Time: 1 h	[57]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
		SLE with ethanol:water Solvent ratio: 1:125 (w/v) RSM Times analyzed: 10–30 min Temperatures analyzed: 25–95°C Solvents concentration: 5–90% Solvent used: food-quality ethanol	[58]
		SLE with methanol:water	[59]
		SLE with acetone:ethanol:water (1:1:1, v/v/v) and with ethanol:water (1:1, v/v) Extraction procedure repetitions: 2 times	[67]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
	Grape pomace (whole pomace)	SLE with water Solvent ratio: 1:10 (w/v) Temperature: 70°C	[68]
		SLE with ethanol:water (80:20, v/v) Solvent ratio: 1:5 (w/v) Extraction temperature: room temperature Extraction time: 48 hours	[69]
		SLE with ethanol with a Soxhlet extractor Solvent ratio: 1:5 (w/v) Extraction time: 105 minutes Extraction temperature: 80°C	[27]
	Vine pruning woods	SLE ethanol:water (50:50, v/v) Solvent ratio: 1:40 (w/v) Extraction temperature: 55°C Extraction time: 2 h	[13]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]

Red grape varieties	Winery byproducts	Extraction conditions	References
Touriga-Franca	Grape stems	SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[66]
		SLE with water, ethanol, ethanol:water (50:50, v/v) Temperature: 45°C Time: 1 h	[57]
		SLE with acetone:ethanol:water (1:1:1, v/v/v) and with ethanol:water (1:1, v/v)	[67]
		SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Grape pomace (whole pomace)	SLE ethanol:water (80:20, v/v) Solvent ratio: 1:5 (w/v) Extraction temperature: room temperature Extraction time: 48 hours	[69]
Tinta-Roriz	Grape stems	SLE with water, ethanol, ethanol:water (50:50, v/v) Temperature: 45°C Time: 1 h	[57]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
		SLE with acetone:ethanol:water (1:1:1, v/v/v) and with ethanol:water (1:1, v/v) Extraction procedure repetitions: 2 times	[67]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
		SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
	Grape pomace (whole pomace)	SLE ethanol:water (80:20, v/v) Solvent ratio: 1:5 (w/v) Extraction temperature: room temperature Extraction time: 48 hours	[69]
		SLE with ethanol with a Soxhlet extractor Solvent ratio: 1:5 (w/v) Extraction time: 105 minutes Extraction temperature: 80°C	[27]
	Vine pruning woods	SLE ethanol:water (50:50, v/v) Solvent ratio: 1:40 (w/v) Extraction temperature: 55°C Extraction time: 2 h	[13]
		SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
		SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]

Red grape varieties	Winery byproducts	Extraction conditions	References
Tempranillo	Grape stems	SLE with ethanol:water (50:50, *v/v*) Solvent ratio: 1:25 (w/v)	[70]
		SLE with ethanol:water (50:50, v/v) Solvent ratio: 1:100 (w/v) Extraction time: 24 h Extraction temperature: 40°C	[71]
	Grape pomace (whole pomace)	SLE with ethanol:water (50:50, v/v) acidified to pH 1.0 with H_2SO_4 Temperature: 60°C	[72]
		SLE using a Soxhlet extractor with ethanol:water (20:80, v/v), ethanol:water (40:60, v/v), ethanol:water (60:40, v/v), ethanol:water (80:20, v/v) Temperature: 120°C SLE with ethanol:water (40:60, v/v) Solvent ratio: 1:8 (w/v) Temperature: 40°C Time: 72 h	[73]
		SLE with ethanol:water (50:50) Solvent ratio: 1:25 (w/v)	[70]
	Grape pomace (seeds)	SLE with ethanol:water (50:50) Solvent ratio: 1:25 (w/v)	[70]
	Wine lees	SLE with ethanol:water (50:50) Solvent ratio: 1:25 (w/v)	[70]
		SLE with ethanol:water (50:50, v/v) acidified to pH 2.5 with HCl Temperature: 25°C	[72]
		SLE with ethanol:water (25:75, v/v), ethanol:water (50:50, v/v), ethanol:water (75:25, v/v) Solvent ratios: 0.1, 0.05, 0.033 and 0.025 g/mL Extraction temperatures: 25°C, 35°C, and 45°C Extraction time: 90 minutes Extraction conditions: pH adjusted to 2.5 with HCl	[74]
		SLE with ethanol:water (50:50, v/v), ethanol:water (75:25, v/v) Solvent ratio: 1:40 (w/v) Extraction temperature: room temperature	[75]
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 180°C The extracts underwent a drying process using a rotary evaporator, after which they were subsequently	[53]

Red grape varieties	Winery byproducts	Extraction conditions	References
		reconstituted in 5 mL of methanol and then added n-hexane to remove nonpolar compounds	
		SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]
Alfrocheiro	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
	Vine pruning woods	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
Jaen	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
	Vine pruning woods	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[65]
Syrah	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
		SLE with deionized water Time: 10 min in the ultrasonic bath	[77]
		SLE with methanol:distilled water mixture (70:30, v/v) Solvent ratio: 1:125 (w/v)	[3]
	Grape pomace (whole pomace)	SLE with methanol Solvent ratio: 1:40 (w/v) pH: 4.0 (addition of HCl)	[78]
		SLE with ethanol proportions (Response Surface Methodology) Solvent ratio: 1:10 (w /v)	[37]
	Grape pomace (seeds, skins)	SLE with methanol acidified (0.1% HCL, v/v) Solvent ratio: 1:15 (w/v) Temperature: 4°C Time: 2 h	[79]
	Grape pomace (seeds, skins)	SLE with ethanol:water (10:90, v/v) pH: 3.5 (tartaric acid)	[80]
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 180°C The extracts underwent a drying process using a rotary evaporator, after which they were subsequently reconstituted in 5 mL of methanol and then added n-hexane to remove nonpolar compounds	[53]
		SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]

Red grape varieties	Winery byproducts	Extraction conditions	References
Merlot	Grape stems	SLE with deionized water Time: 10 min in the ultrasonic bath	[77]
		SLE with methanol:water (80:20, v/v) Time: 3 h	[81]
	Grape pomace (whole pomace)	SLE with methanol acidified with 0.1% HCl Solvent ratio: 1:50 (w/w) Extraction condition: powdering in liquid nitrogen Extraction temperature: - 4°C Extraction time: 1 hour (4 x 15 min)	[82]
		SLE with methanol:water (80:20, v/v), ethanol:water (80:20, v/v), acetone (100:0, v/v), ethyl acetate (100:0, v/v), methanol: water (50:50, v/v) + acid, methanol:water (80:20, v/v) + acid Solvent ratio: 1:10 (w/v) Extraction temperature: room temperature Extraction condition: removal of nonpolar compounds with petroleum ether Extraction time: 6 h	[83]
		SLE with ethanol:water (70:30, v/v) Temperature: 30°C	[84]
	Wine lees	SLE with methanol:water:formic acid (80:18.5:1.5, v/v/v) Solvent ratio: 1:1 (w/v)	[85]
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]
Tinto-Cão	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[62]
Tinta-Barroca	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[62]
		SLE with methanol:water	[59]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Sousão	Grape stems	SLE with methanol:water	[59]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
		SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[66]

Red grape varieties	Winery byproducts	Extraction conditions	References
Tinta-Amarela	Grape stems	SLE with methanol:water	[59]
		SLE with methanol:water (70:30, *v/v*) Solvent ratio: 1:125 (w/v)	[66]
		SLE with methanol:formic acid:water (50:2:48, v/v/v) Solvent ratio: 1:125 (w/v)	[61]
	Vine pruning woods	SLE with five different ratios of ethanol:water, namely 0:100, 25:75, 50:50, 75:25, and 100:0 (v/v) Solvent ratio: 1:125 (w/v)	[56]
Castelão	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
Cabernet-Sauvignon	Grape stems	SLE with deionized water Time: 10 min in the ultrasonic bath	[77]
		SLE with ethanol:water (50:50, v/v) Solvent ratio: 1:100 (w/v) Extraction time: 24 h Extraction temperature: 40°C	[71]
	Grape pomace (whole pomace)	SLE with an aqueous solution of base Na_2CO_3 2.5% (w/w) and Na_2SO_3 2.5% (w/w) based on dry pomace Temperature: 100°C Time: 120 min	[33]
		SLE with methanol acidified with 0.1% HCl Solvent ratio: 1:50 (w/w) Extraction condition: powdering in liquid nitrogen Extraction temperature: - 4°C Extraction time: 1 hour (4 x 15 min)	[82]
		SLE with methanol:water (80:20, v/v), ethanol:water (80:20, v/v), acetone (100:0, v/v), ethyl acetate (100:0, v/v), methanol: water (50:50, v/v) + acid, methanol:water (80:20, v/v) + acid Solvent ratio: 1:10 (w/v) Extraction temperature: room temperature Extraction condition: remotion of nonpolar compounds with petroleum ether Extraction time: 6 h	[83]
		SLE with ethanol in deionized water at 0, 20, 40, 60, 80, and 100% concentrations (v/v) Extraction time: 90 minutes	[86]
		SLE with methanol:water:acetic acid (80:20:5, v/v/v) Solvent ratio: 1:50 (w/v)	[87]
		SLE with methanol acidified (0.1% HCl, v/v)	[79]

Red grape varieties	Winery byproducts	Extraction conditions	References
		Solvent ratio: 1:15 (w/v) Temperature: 4°C Time: 2 h	
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 180°C The extracts underwent a drying process using a rotary evaporator, after which they were subsequently reconstituted in 5 mL of methanol and then added n-hexane to remove nonpolar compounds	[53]
		SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]
Sauvignon-Blanc	Grape stems	SLE with water Solvent ratio: 1:25 (w/v) Extraction temperature: 348 Kelvin (74.85°C) Extraction time: 1.25 h	[88]
		SLE with 75% methanol Extraction time: 2 h Extraction condition: addition of 75% sulfur dioxide to prevent oxidation	[89]
	Grape pomace (whole pomace)	SLE with water Solvent ratio: 1:25 (w/v) Extraction temperature: 348 Kelvin (74.85°C) Extraction time: 1.25 h	[88]
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 180°C The extracts underwent a drying process using a rotary evaporator, after which they were subsequently reconstituted in 5 mL of methanol and then added n-hexane to remove nonpolar compounds	[53]
		SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]
Chardonnay	Grape stems	SLE with ethanol:water (50:50, v/v) Solvent ratio: 1:100 (w/v) Extraction time: 24 h Extraction temperature: 40°C	[71]
	Grape pomace (whole pomace)	SLE with an aqueous solution of base Na_2CO_3 2.5% (w/w) and Na_2SO_3 2.5% (w/w) based on dry pomace Temperature: 100°C Time: 120 min	[33]
	Vine pruning woods	SLE with ethanol:water (80:20, v/v) at pH 3 Extraction time: 1 hour Extraction temperature: 220°C	[76]

Red grape varieties	Winery byproducts	Extraction conditions	References
Pinot-Noir	Grape pomace (whole pomace)	SLE with methanol:water:acetic acid (80:20:5, v/v/v) Solvent ratio: 1:50 (w/v)	[87]
		SLE with an aqueous solution of base Na_2CO_3 2.5% (w/w) and Na_2SO_3 2.5% (w/w) based on dry pomace Temperature: 100°C Time: 120 min	[33]
		SLE with ethanol:water:formic acid (50:48.5:1.5, v/v/v) Solvent ratio: 1:7.5 (w/v)	[90]
	Wine lees	SLE with ethanol:water:formic acid (50:48.5:1.5, v/v/v) Solvent ratio: 1:7.5 (w/v)	[90]
Merlot	Grape stems	SLE with methanol:water (70:30, v/v) Solvent ratio: 1:125 (w/v)	[64]
	Grape pomace (whole pomace)	SLE with methanol acidified (0.1% HCL, v/v) Solvent ratio: 1:15 (w/v) Temperature: 4°C Time: 2 h	[79]

SLE, Solid–Liquid Extraction; RSM, Response Surface Methodology.

Table 2.
Studies performed on the extraction of phenolic compounds from WBPs of red grapes (Vitis vinifera L.*) varieties using conventional methodologies.*

toxicity, lower extraction efficiency, and concerns regarding environmental disposal [12, 48, 91]. These innovative technologies harbor significant potential, particularly when extracting high-value compounds from WBPs.

These modern methods that adhere to the principles of green chemistry and ecological practices have gained special attention for improving the extraction of phenolic compounds. According to the literature, these methods are Ultrasound Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Pressurized Solvent Extraction (PSE), Enzyme Assisted Extraction (EAE), High Hydrostatic Pressure (HHP), High Voltage Electrical Discharges (HVED), Pulsed Electric Fields (PEF), and Surfactant Mediated Extraction (SME).

UAE harnesses the principle of acoustic cavitation. This involves the generation of mechanical energy through cycles of compression and rarefaction, transmitted via ultrasonic waves to generate nanobubbles. As these nanobubbles' energy surpasses their resistance threshold, they collapse, rupturing plant cell walls and enabling solvent penetration within the cells. Consequently, there is a bigger transfer of bioactive compounds [92]. This extraction method offers numerous advantages, including simplicity of use, efficiency, rapidity, selectivity, energy efficiency, economic benefits, and high extraction efficiency. This extraction method is advantageous for thermolabile compounds, as it does not require high temperatures. However, it may lead to liquid oxidation and the formation of free radicals [12, 92]. While this extraction method has primarily found application in laboratory settings, it has also been

increasingly utilized across diverse industrial sectors [93, 94]. In the study of Alexandru et al. [95], they reported that the use of this technique enhanced phenolics recovery and antioxidant capacity compared to maceration in grape pomace and grapevine shoots.

MAE uses microwave energy to disrupt the hydrogen bonds of polar molecules within the extraction system, rapidly rotating them via ion conduction or dipole-dipole rotation. Furthermore, it is highly recommended for extracting short-chain polyphenols like flavonoids and phenolic acids from plant materials [92] and is suitable for thermolabile phenolic compounds [12]. Highlighting its advantages, it offers simplicity, short extraction times, and low consumption of both solvent and energy. On the other hand, it requires careful selection of power to prevent excessive temperatures. Factors such as extraction time, temperature, the irradiation power, and the dielectric constant of the solvent can influence the polyphenol extraction [12, 92]. This environmentally friendly extraction method has also been applied in several industrial sectors [94]. This technique enhanced the recovery of polyphenols from grape pomace, as shown by Álvarez et al. [96], which increased yields by 57% and anthocyanin content by 85%. When compared to conventional maceration, the adoption of this approach improved phenolics recovery and antioxidant capacity, according to Alexandru et al. [95]. Moreira et al. [13] demonstrated in their study with grapevine shoots that this extraction technique led to a higher phenolic content compared to SLE and Sub Critical Water Extraction (SWE).

PSE, also referred to as Pressurized Liquid Extraction (PLE) or Accelerated Solvent Extraction (ASE). If water is employed as the solvent, it is alternatively referred to as Pressurized Hot Water Extraction (PHWE), Sub Critical Water Extraction (SWE), or Superheated Water Extraction (SHWE) [48, 91]. This methodology is based on a pressurized extraction chamber (10–15 MPa), which keeps the solvent in a liquid state at a temperature exceeding its boiling point (up to a maximum of 200°C) and under higher pressures (approximately 1700 psi). Subsequently, the extraction efficiency is boosted through enhanced mass transfer and solubility of compounds within the medium [48]. It offers the advantages of faster extraction compared to conventional techniques and low solvent use. However, it exhibits low selectivity, requires high temperatures, and entails costly equipment. Its primary application is the extraction of phenolic compounds [12]. Luque-Rodríguez et al. [43] employed this methodology to extract phenolics from vine shoots, demonstrating increased yield and reduced extraction time compared to SLE. Moreira et al. [13] also used this technique to compare SLE, MAE, and SWE, concluding that SWE resulted in the highest flavonoid content.

EAE is a sophisticated extraction method leveraging enzymes such as cellulases, hemicelluloses, pectinases, and other enzymes that can be used to catalyze the hydrolysis of the polysaccharides in the cell wall, which is primarily composed of celluloses, hemicelluloses (xyloglucans), pectin, and proteins, thereby enabling better release and extraction of phenolic compounds. These compounds are bound to cell wall polysaccharides through hydrophobic interactions and hydrogen bonds [97].

HHP involves the application of high pressures (namely 100–1000 MPa) to a matrix, transmitted by a liquid within a closed system. In this green extraction, pressure forces air out of plant cell vacuoles, leading to cell membrane damage and improved contact with the extraction solvent. Additionally, it can modify the conformation or denature cell membrane proteins, decreasing their selectivity and thus making phenolic compounds more accessible for extraction. The pressure transmitter fluid is generally water, and the process can be used with or without the utilization of

temperature. Nowadays, a large number of studies have focused on innovative applications of HHP, such as the improvement of polyphenols' extraction [98, 99].

HVED includes electrical breakdown, which is accompanied by various secondary phenomena, such as high-amplitude pressure shock waves, bubble cavitation, liquid turbulence generation, and active species production, leading to particle fragmentation and damage to the cellular structure, thereby facilitating the release of intracellular compounds [100, 101]. This method has shown promising results in laboratory studies [93].

PEF implies placing the sample between two electrodes, varying the pulse amplitude between 100 and 300 V/cm and 20–80 kV/cm, conducted at room temperature or slightly higher. Plant cells are exposed to a certain electric field, and consequently, cell membranes can be damaged, leading to the formation of temporary or permanent pores [54, 98, 101].

SME is a technique for extracting substances from a complex matrix, such as plant materials or soil, employing surfactants. These surfactants are compounds characterized by possessing both hydrophilic (water-attracting) and hydrophobic (water-repelling) properties within their molecular structure and can exist as neutral compounds (non-ionic) or as part of anionic or cationic systems [102]. With this type of extraction, surfactants are used to aid in the solubilization and extraction of target substances from the matrix through the formation of micelles or other complexes, where the hydrophilic portion of the surfactant interacts with water molecules, while the hydrophobic portion interacts with the nonpolar compounds of interest [15, 102, 103]. It offers several advantages, including better efficiency of polyphenol extraction, and is recognized as relatively nontoxic, with desirable stability and compatibility, including those that are poorly soluble in traditional solvents. Additionally, it can be a more environmentally friendly option compared to organic solvent-based extraction methods, as surfactants are often biodegradable and can reduce the need for toxic organic solvents [15, 103].

Tables 3 and **4** show several studies on the extraction of phenolic compounds using nonconventional methodologies using different extraction conditions from WBPs of white and red grape varieties, respectively, considering solvent ratios, temperatures, extraction times, and other variables.

White grape varieties	Winery byproducts	Extraction conditions	References
Sauvignon-Blanc	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3	[53]

White grape varieties	Winery byproducts	Extraction conditions	References
		Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	
Chardonnay	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
		ASE (ASE 350) system equipped with a solvent controller Extraction solvents: acetone:water (80:20, v/v), methanol:water (60:40, v/v) Static time: 4 min Pressure: 1500 psi Temperature: 40°C Heating period: 5 min	[105]
	Grape pomace	MAE Optimal conditions using RSM: Extraction solvent: ethanol:water (48:52, v/v) Time: 10 min Solid mass: 1.77 g	[106]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]

ASE, Accelerated Solvent Extraction; MAE, Microwave Assisted Extraction; SHLE, Superheated Liquid Extraction; UAE, Ultrasound Assisted Extraction; RSM, Response Surface Methodology.

Table 3.
Studies performed on the extraction of phenolic compounds from WBPs of white grapes (Vitis vinifera L.*) varieties using unconventional methodologies.*

Red grape varieties	Winery byproducts	Extraction conditions	References
Tempranillo	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
		ASE (ASE 350) system equipped with a solvent controller Extraction solvents: acetone:water (80:20, v/v), methanol:water (60:40, v/v) Static time: 4 min Pressure: 1500 psi Temperature: 40°C Heating period: 5 min	[105]
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
	Grape pomace	UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
		MAE (Used as a pretreatment) Extraction solvent: ethanol:water (50:50, v/v) Time of irradiation: 60 s Temperature: 80°C Power: 300 W	[72]
	Wine lees	UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
		MAE (Used as a pretreatment) Extraction solvent: ethanol:water (60:40, v/v) Time of irradiation: 90 s Temperature: 115°C Power: 300 W	[72]
		MAE Extraction solvent: ethanol:water (60:40, v/v) adjusted to pH 4 with formic acid Irradiation power: 140 W for 10 minutes Solvent ratio: 1:8.3	[107]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3	[53]

Red grape varieties	Winery byproducts	Extraction conditions	References
		Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	
Syrah	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
		ASE (ASE 350) system equipped with a solvent controller Extraction solvents: acetone:water (80:20, v/v), methanol:water (60:40, v/v) Static time: 4 min Pressure: 1500 psi Temperature: 40°C Heating period: 5 min	[105]
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
		UAE Extraction solvent: ethanol:water (80:20, v/v) Solvent ratio: 1:30 Temperature: 75°C Time: 15 min Amplitude: 70% Cycle: 0.7 s	[108]
	Grape pomace	UAE Extraction solvent: water Solvent ratio: 1:20 (w/v) Temperatures: 20, 35, 50°C	[109]
		UAE Extraction solvent: water Solvent ratio: 1:5 (w/v) Acoustic frequency: 40, 80, 120 kHz Ultrasonic power density: 50, 100, 150 W/L Times: 5, 15, 25 min	[110]
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
	Wine lees	MAE Extraction solvent: ethanol 75% (hydrochloric acid 1% in water)	[111]

Red grape varieties	Winery byproducts	Extraction conditions	References
		Irradiation power: 200 W for 17 min Solvent ratio: 1:10 (w/v)	
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25(w/v) Time: 15 min	[32]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]
Merlot	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
		ASE (ASE 350) system equipped with a solvent controller Extraction solvents: acetone:water (80:20, v/v), methanol:water (60:40, v/v) Static time: 4 min Pressure: 1500 psi Temperature: 40°C Heating period: 5 min	[105]
		PLE Extraction solvent: ethanol:water mixtures (0–100%) Pressure: 1500 psi Temperatures tested: 40–120°C Times tested: 1–11 min Optimal conditions using RSM: 30% ethanol: water, 120°C, 10 min	[22]
	Grape pomace	UAE Extraction solvent: methanol acidified with 2% formic acid Solvent ratio: 1/100 (w/v) Ultrasonication: 59 kHz Time: 10 min Temperature: 25–35°C	[112]
	Wine lees	UAE Extraction solvent: aqueous ethanol solution	[113]

Red grape varieties	Winery byproducts	Extraction conditions	References
		Frequency: 40 kHz Acoustic energy density: 48 W/L	
		UAE Extraction solvent: ethanol:water:formic acid (50:48.5:1.5, v/v/v) at pH 2.7 Different times and ultrasonic powers were tested depending on the experimental design	[114]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]
Cabernet-Sauvignon	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
		ASE (ASE 350) system equipped with a solvent controller Extraction solvents: acetone:water (80:20, v/v), methanol:water (60:40, v/v) Static time: 4 min Pressure: 1500 psi Temperature: 40°C Heating period: 5 min	[105]
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
	Grape pomace	ASE Extraction solvent: ethanol:water (70:30, v/v) Optimal conditions using RSM: 140°C	[115]
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
	Wine lees	UAE Extraction solvent: aqueous ethanol solution	[113]

Red grape varieties	Winery byproducts	Extraction conditions	References
		Frequency: 40 kHz Acoustic energy density: 48 W/L	
		UAE Extraction solvent: methanol Solvent ratio: 1:0.25 (w/v) Time: 15 min	[32]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]
Cabernet-Franc	Grape pomace	SME Solvent/liquid ratios: 1:10, 1:20, 1:100 (*w/v*) pH: 4.0 Time: 45 min	[15]
	Wine lees	UAE Extraction solvent: aqueous ethanol solution Frequency: 40 kHz Acoustic energy density: 48 W/L	[113]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]
Lemberger	Grape pomace	EAE Extraction solvent: water Solvent ratio: 1:3 (w/w) Enzymes: Novoferm 106, Cellubrix L Temperature: 50°C Time: 2 h pH: 4.0	[116]
		EAE Extraction solvent: water Solvent ratio: 1:3 (w/v) Temperature: 80°C Enzymes: pectinolytic and cellulolytic enzymes	[30]

Red grape varieties	Winery byproducts	Extraction conditions	References
Chambourcin	Grape pomace	PLE Extraction solvent: ethanol:water (40:60, v/v) Tested conditions using RSM: Temperatures: 70, 100, 130°C Extraction times: 1, 5, 9 min Ethanol concentrations: 20, 50, 80% Optimal conditions: Time: 9 min Temperature: 130°C Extraction procedure repetitions: 5 times	[29]
Petit-Verdot	Grape stems	UAE Extraction solvent: ethanol:water (50:50, v/v) Extraction temperatures: 5–65°C Ultrasound amplitude: 30–70% Ultrasonic cycle duration: 0.2–0.7 s Ultrasonic probe tip: 2–7 mm Extraction time: 5–15 min Solvent ratios: 1:25, 1:50 Intensity: 200 W and 24 kHz	[104]
	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]
Cabernet-Mitos	Grape pomace	EAE Extraction solvent: water Solvent ratio: 1:3 (w/v) Temperature: 80°C Enzymes: pectinolytic and cellulolytic enzymes	[30]
Alicante Bouschet	Grape pomace	HHP Extraction solvent: sodium acetate buffer (pH 5) Solvent ratio: 1:8 (w/v) Times: 0, 5, 10, 15, 30 min, and 2 h	[99]
Malbec	Vine pruning woods	SHLE Solvent extraction: ethanol:water 80:20 (v/v) at pH 3.60 Extraction temperature: 180°C Extraction time: 60 min MAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Solvent ratio: 1:20 (w/v) Extraction time: 5 min Irradiation power: 140 W UAE Extraction solvent: ethanol:water 80:20 (v/v) at pH 3 Irradiation power: 280 W	[53]

Red grape varieties	Winery byproducts	Extraction conditions	References
Nebbiolo	Vine pruning woods	UAE Extraction solvent: ethanol with 1.5% β-cyclodextrin solution for 5 min at 100 W and 30 min at 80 W Solvent ratio: 1:10 (w/v) MAE Extraction solvents: ethanol, ethanol:water (50:50, v/v), acetone, butanone Temperature: 60°C Time: 30 min Power: 1.5 kW under nitrogen pressure (5 bar)	[95]
Grenache	Vine pruning woods	PEF, HVED, and UAE (Used as a pretreatment) Extraction solvent: basified water (0.1 M of NAOH) Temperature: 50°C	[100]
Tinta-Roriz	Vine pruning woods	MAE Extraction solvent: ethanol:water (60:40, v/v) Solvent ratio: 1:200 (w/v) Extraction temperature: 100°C Extraction time: 20 min SWE Solvent ratio: 1:40 (w/v) Extraction temperature: 150°C Extraction time: 40 min Pressure: 40 bars Frequency: 3 Hz	[13]
Touriga-Nacional	Vine pruning woods	MAE Extraction solvent: ethanol:water (60:40, v/v) Solvent ratio: 1:200 (w/v) Extraction temperature: 100°C Extraction time: 20 min SWE Solvent ratio: 1:40 (w/v) Extraction temperature: 150°C Extraction time: 40 min Pressure: 40 bars Frequency: 3 Hz	[13]

EAE, Enzyme Assisted Extraction; HHP, High Hydrostatic Pressure; HVED, High Voltage Electrical Discharges; MAE, Microwave Assisted Extraction; PEF, Pulsed Electric Fields; PLE, Pressurized Liquid Extraction; RSM, Response Surface Methodology; SWE, Subcritical Water Extraction; UAE, Ultrasound Assisted Extraction.

Table 4.
Studies performed on the extraction of phenolic compounds from WBPs of red grapes (Vitis vinifera L.*) varieties using unconventional methodologies.*

4. Challenges, limitations, and future directions

This review evidenced that grape pomace is the WBP with the highest number of studies comparing the different extraction methods. In contrast, grape stems remain the least explored, particularly when it comes to unconventional extraction

methodologies. In this regard, further research is necessary on the extraction of these phytochemicals present in grape stems using nonconventional methods.

WBPs hold significant potential for use in several added-value products due to their content of bioactive compounds, namely phenolic compounds. However, the choice of extraction method directly influences the recovery of these valuable compounds present in WBPs.

Furthermore, the extracted phenolic compounds face challenges related to low bioavailability, chemical instability, low solubility, occasional low extraction yields, low stability, and sensitivity to environmental factors such as light and heat, resulting in loss of their bioactivity [27, 117, 118]. For these reasons, more research is needed to increase the extraction yields while reducing the processing time of these valuable compounds [119]. The adoption of green technologies, replacing toxic solvents with more sustainable alternatives, is essential to optimizing processing [119].

The study by Liu et al. [120] demonstrated that UAE and SLE extracted a similar number of phenolic acids, though with different compositions. However, the UAE allows the identification of a higher number of flavonoids compared to the conventional method, suggesting that this nonconventional approach efficiently extracts flavonoids in a shorter time while maintaining a similar chemical composition to SLE [120]. The same authors also concluded that the UAE holds significant potential for green, large-scale industrial production, helping to reduce both economic and environmental impacts. In this sense, combining UAE with SLE or other techniques [121] could be a promising strategy to enhance extraction yields and improve the recovery of phenolic compounds. For example, in the study conducted by Matos et al. [122], which focused on extracting phenolics from wine lees, the microwave pretreatment prior to conventional SLE led to increased total phenolic content, total anthocyanin content, and enhanced antioxidant activity. A similar outcome was observed in the study by Rajha et al. [100], where HVED, PEF, and UAE pretreatment increased the extraction efficiency of total polyphenols, kaempferol, epicatechin, and resveratrol compared to untreated grapevine shoot samples. Romero et al. [121] also utilized microwave pretreatment and enhanced anthocyanin extraction yield, reducing processing time in wine lees samples. For easy scale-up and cost-effective production, simple and effective extraction and purification procedures should be chosen in order to enhance the recovery of bioactive compounds, maintain their integrity, and maximize their potential for commercialization [93].

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Conflict of interest

The authors declare no conflict of interest.

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Section 2

Technological Innovations and Functional Applications

Chapter 5

Enhancing Polyphenol Bioavailability through Nanotechnology: Current Trends and Challenges

Hanae El Monfalouti and Badr Eddine Kartah

Abstract

Polyphenols are a class of plant secondary metabolites that have increasingly been gaining traction due to their multiple roles as therapeutics, food supplements, and preservatives. They are widely used in various foods to enhance flavor, texture, shelf life, and overall quality. Polyphenols are efficient antioxidants and radical scavengers with significant health benefits, including anti-inflammatory and antimicrobial effects. Several studies demonstrated that an increased consumption of polyphenol-rich foods may help reduce the risk metabolic disorders and cancer. However, their bioavailability is limited after ingestion due to low water solubility, instability at low pH values, and difficulties for absorption in the small intestine. To address these challenges, new technological processes and the use of nanoparticles loaded with polyphenols encapsulation and nanotechnology are required to improve polyphenol bioavailability and to maintain their biological activities, making them more effective as functional food ingredients and drug delivery systems. This chapter covers the latest trends and innovative techniques in polyphenol-based nanotechnology and explores the challenges associated with their use in these applications.

Keywords: polyphenols, nanoparticles, bioavailability, encapsulation, nanotechnology

1. Introduction

Polyphenols are a class of chemical components found naturally in various plants, including vegetables, fruits, and cereals. They are characterized by complex structures, including phenolic acids, flavonoids, anthocyanins, and tannins, which can be isolated directly from natural sources or obtained through processing.

Polyphenols are antioxidants that possess anti-inflammatory and anticancer properties due to their chemical structure [1]. They have the potential to prevent chronic and degenerative diseases, including cardiovascular, cancer, liver, and neurological diseases [2, 3].

Despite their wide range of biological effects, polyphenols face degradation challenges after extraction due to factors such as pH and oxygen [4]. The variation

in postconsumer circulation can be attributed to several factors, particularly the low solubility in water and sensitivity to light, heat, and environmental conditions during processing [5]. Additionally, the suboptimal bioavailability of polyphenols is influenced by various factors, including harvest conditions, food processing methods, and interactions between compounds and host factors [6]. Furthermore, the limited solubility of these compounds in body fluids and their rapid metabolism *in vivo* significantly impact their overall bioavailability [7]. This restricts the use of many of these compounds in nutraceuticals and as therapeutic agents. The bioavailability of polyphenols is influenced by several factors, such as molecule dimensions, degree of polymerization, the presence and type of sugar in the molecule, and their hydrophobicity.

This chapter examines the potential of novel delivery systems to enhance the bioavailability of polyphenols. Nanotechnology is a promising avenue for investigation, given the challenges posed by polyphenol's low solubility and rapid metabolism. Nanocarriers, such as nanoparticles, liposomes, and nanoemulsions, have the capacity to encapsulate and protect polyphenols from degradation in the gastrointestinal tract, thereby improving their bioavailability.

Furthermore, nanotechnology can be employed to deliver polyphenols to specific tissues or cells, thereby enhancing therapeutic efficacy while reducing the incidence of systemic side effects.

2. General aspects of polyphenols

2.1 Classification and properties of plant polyphenols

Polyphenols represent a diverse group of phytochemicals that are abundant in fruits and vegetables. These compounds play an essential role in plant adaptation to stressors such as infection, UV exposure, or injury. They comprise over 8000 phenolic structures that are synthesized *via* pathways such as the pentose phosphate and shikimate phenylpropanoid structures [8, 9]. These compounds, which are characterized by the presence of one or more phenolic hydroxyl groups, represent form the primary heterogeneous group of secondary metabolites in plants [10, 11]. Polyphenols exist in free or bound forms, with free phenolics being readily soluble in water or organic solvents and bound phenolics being covalently bound to other molecules (**Figure 1**).

Phenolic compounds exist in conjugated form with one or more sugar molecules, forming glycosides linked by hydroxyl (OH) groups (O-glycosides) or carbon-carbon bonds (C-glycosides). These sugar linkages can be monosaccharides, disaccharides, or even oligosaccharides, with glucose being the most common. However, it can also bind to galactose, rhamnose, arabinose, xylose, or glucuronic acid [13].

The concentration and nature of polyphenols in plant foods vary depending on a number of factors, including plant genetics, growing conditions, soil composition, harvest maturity, and postharvest handling [14]. These compounds, which include phenolic acids, flavonoids, stilbenes, and lignans, have different chemical structures and can be broadly categorized as flavonoids and non-flavonoids [15].

Flavonoids, which are found in a wide variety of plant foods, play an important role in plant growth, development, flowering, fruiting, and the vibrant colors of fruit and vegetables. They form a central category of polyphenols, sharing a common diphenylpropane structure (C6-C3-C6). This group is further subdivided into eight primary subclasses, defined by variations in the heterocyclic ring. These subclasses

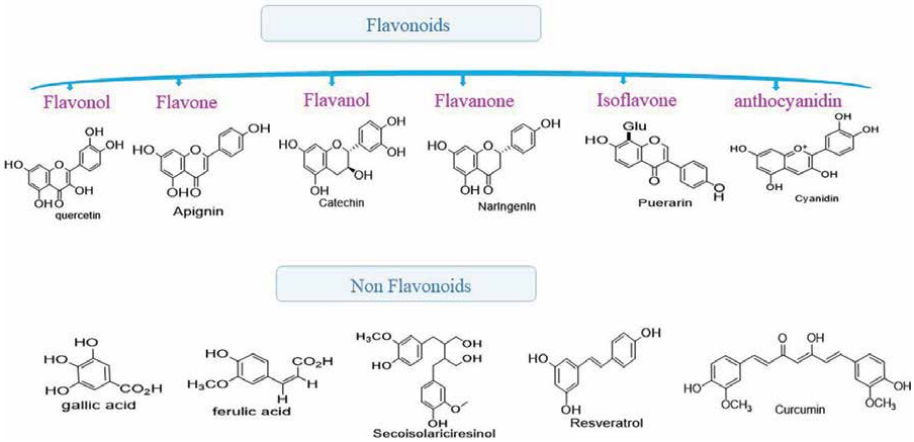

Figure 1.
Chemical structure of the main polyphenols and their biological relevance [12].

include flavonols, flavones, flavanones, isoflavones, flavanols, anthocyanins, proanthocyanidins, and tannins [16]. Quercetin and kaempferol, well-known members of the flavonol group, are often found in glycosylated forms, which increases their solubility and stability.

Anthocyanins are water-soluble pigments that impart blue, purple, and red hues characteristic of fruits and vegetables. These pigmented compounds are glycosides of anthocyanidins, a structural class that renders them unstable in their native form. However, attachment of glucose molecules stabilizes them.

Non-flavonoids are composed of one or two aromatic rings and are classified as phenolic acids, which contain a C6-C1 carbon skeleton, hydroxycinnamates with a structure of C6-C3, hydrolysable tannins with one or two aromatic rings, and stilbenes, with a more complex structure of C6-C2-C6 [17, 18].

Phenolic acids are the most abundant non-flavonoid polyphenols, and they are found in a wide range of plant foods, such as coffee, tea, and fruits. These compounds possess diverse carbon backbones and hydroxyl group arrangements. Hydroxybenzoic acids are derived from benzoic acid, while hydroxycinnamic acids such as caffeic acid and ferulic acid contribute to the phenolic acid category. These compounds play a multifunctional role in foods, assisting in the retention of color, the inhibition of microbial growth, and the prevention of lipid oxidation, thereby extending the shelf life of foods [19].

Stilbenes are characterized by the presence of a 1,2-diphenylethylene nucleus with hydroxyl substituted on the aromatic rings and exist in the form of monomers or oligomers [20]. The main compound representative of stilbenes is resveratrol, which is primarily concentrated in grapes, berries, and peanuts. Resverastrol has been demonstrated to exert immune-boosting, anti-inflammatory, and anti-angiogenic effects.

2.2 Beneficial effects of polyphenols

Plant polyphenols offer a wide range of health benefits to humans. Their remarkable biological activities, including antioxidant and antibacterial properties, combined with their natural availability and compatibility with the human body, make them valuable additions to foods, giving them unique functional properties that promote human health. As antioxidants, polyphenols play a crucial role in the prevention of various

diseases by neutralizing free radicals and protecting DNA from oxidative damage [21]. A study by Grzesik et al. [22] demonstrated that the antioxidant capacities of catechins were found to be particularly noteworthy, exhibiting superior efficacy in scavenging ABTS radicals and protecting against oxidative damage. These properties position polyphenols such as catechins as promising candidates for antioxidant therapy and prophylaxis. Similar effects were observed with a grape seed extract rich in catechins, proanthocyanidins, and anthocyanidins in the human keratinocyte cell line HaCaT. The antioxidant activity of the extract shielded keratinocytes from ROS formation; mitigated oxidative stress, DNA damage, and apoptosis; and increased cell survival [23].

Polyphenols have been found to have significant anticancer properties by inhibiting tumor growth and inducing apoptosis in malignant cells. Flavonoids regulate the activity of ROS-scavenging enzymes, participate in cell cycle arrest, induce apoptosis and autophagy, and suppress cancer cell proliferation and invasion [24]. A study conducted by Lee et al. [25] highlights the important roles of compounds such as resveratrol and quercetin in impeding cancer progression through various mechanisms, including apoptosis and antioxidant capabilities. Furthermore, silymarin has been demonstrated to induce apoptosis in liver cancer cells, indicating promising preventive and therapeutic effects against liver diseases. Similarly, epigallocatechin gallate (EGCG) and curcumin have demonstrated significant anticancer effects on breast cancer [26, 27]. Additionally, research has shown that phenolic acid can inhibit the activation of the tumor protein p53, which can enhance the effectiveness of conventional chemotherapy [28, 29].

Resveratrol, a stilbenoid polyphenol found in whole grains, exerts a neuroprotective effect in 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease by reducing DNA condensation and vacuolization of dopaminergic neurons in the substantia nigra, as shown in the study by Bhullar and Rupasinghe [30]. In a noteworthy clinical trial, daily consumption of anthocyanin-rich cherry juice showed significant benefits in older adults with Alzheimer's disease. Improvements were observed in verbal fluency, short-term memory, long-term memory, and a reduction in both systolic and diastolic blood pressures [31].

Other beneficial effects have been attributed to various polyphenols. Ding et al. [32] have demonstrated the anti-inflammatory potential of hesperidin, highlighting its effectiveness in reducing nitric oxide (NO), interleukin (IL-6), and tumor necrosis factor (TNF-α) levels both *in vitro* and *in vivo*. These findings underscore the valuable anti-inflammatory properties of certain polyphenols.

Phenolic compounds can modulate lipid metabolism, inhibit low-density lipoprotein (LDL) oxidation, increase high-density lipoprotein (HDL) levels, induce vasodilation, and reduce the risk of coronary diseases, ischemia, and cardiomyopathies. They also inhibit platelet aggregation, enhance endothelial function, and decrease the expression of cell adhesion molecules [33].

Phenolic acids have been identified as having a multitude of beneficial effects on human health, including anti-inflammatory, anti-allergenic, antidiabetic, immunoregulatory, and cardioprotective properties [34]. Studies have demonstrated that plant-derived phenolic acids possess tyrosinase-inhibiting activity, which can be exploited to treat UV light-induced skin hyperpigmentation [35–39].

3. Bioavailability of polyphenols

Polyphenols have gained significant attention due to their potential health benefits. However, the bioavailability of polyphenols is limited due to their low solubility

and uptake in the gastrointestinal tract. Additionally, polyphenols are susceptible to degradation in light, heat, and alkaline environments due to their numerous -OH groups [40]. Studies have shown that only a small fraction of dietary polyphenols can be absorbed and reach their target cells to exert their biological effects. To fully exploit the potential health benefits of polyphenols, it is crucial to comprehend their bioavailability.

Bioavailability, in conjunction with the polyphenol content in foods and their distribution in plants, represents a pivotal factor that directly influences and determines the biological function of consuming polyphenol-rich foods. It is crucial to distinguish between bioaccessibility and bioavailability.

Bioaccessibility is defined as the proportion of a food substance that reaches the gastrointestinal tract, where it is subjected to digestion, absorption by intestinal epithelial cells, and undergoes metabolic transformations in the intestine and liver [41]. In general, bioaccessible phenolic compounds make up less than 30% of the total phenolic content in vegetables, fruits, and nuts before digestion. In some cases, the bioaccessibility can reach up to 50% of the total polyphenol content [42]. In contrast, bioavailability refers to the release of a bioactive compound from the food matrix, its subsequent digestion, absorption, metabolism in the liver and intestine, and distribution to target tissues or storage in human cells, cultures, or organs where it exerts its bioactivity [43]. Therefore, any condition that affects the bioaccessibility of a bioactive compound directly affects its bioavailability.

The bioavailability, absorption, and metabolism of polyphenols are profoundly influenced by the chemical structure of the compounds and the diversity of species and genera of the gut microbiota. In addition to intestinal factors, the biochemical transformations of polyphenols and the types and proportions of their derivative metabolites depend on systemic host factors such as sex, age, presence of pathologies, and genetics [44].

During gastric digestion, most phenolic compounds resist acidic conditions [45]. Biotransformation processes, such as deglycosylation, also play a critical role in determining bioavailability. Many polyphenols (aglycones) are hydrophilic and can diffuse across biological membranes for absorption, while glycosylated polyphenols (glycons) require hydrolysis of linked sugar groups by intestinal enzymes or colonic microflora prior to absorption [46, 47].

The bioavailability of polyphenols is intricately linked to the different pH levels and enzymatic activities present in the gastrointestinal fluids (GIF) along the digestive tract. The acidic environment of the stomach (pH 1.2) and the more neutral conditions of the small intestine (pH 6.8), along with various gastrointestinal tract (GIT) metabolizing enzymes, exert a significant influence on the fate of orally administered polyphenols. Consequently, numerous polyphenols are susceptible to degradation in the acidic environment of the stomach and enzymatic breakdown in the gastrointestinal tract, resulting in reduced bioavailability [48]. Intestinal absorption of polyphenols is strongly influenced by their chemical structure and the nature of the sugars in their glycosylated form [49]. Typically, polyphenols are glycosylated and the attached sugars are usually released prior to absorption. Several dietary compounds, including dietary fiber, lipids, proteins, and digestible carbohydrates, can modulate the availability of polyphenols for absorption after ingestion. Furthermore, the ability of polyphenols to bind to proteins represents a significant limitation to their absorption.

During digestion, complex polyphenols are broken down into simpler molecules by several enzymes. Insoluble complexes, such as those composed of phenolic moieties and indigestible polysaccharides [50], protein [51], and other dense polyphenols,

including condensed tannins and lignins [52], are also present. The bioavailability of polyphenols varies. Isoflavones have the highest bioavailability, followed by phenolic acids, flavanols, flavanones, flavonols, anthocyanins, and proanthocyanidins [53]. The absorption of hydrophobic compounds such as curcumin and naringenin is low due to rapid metabolism and excretion [54]. Anthocyanins are known to be unstable, particularly during food processing and exposure to gastrointestinal diseases, which can limit their absorption [53].

Polyphenols that are not absorbed in the small intestine are transferred to the colon, where they are converted by the colonic microflora into other bioactive phenolic metabolites. These metabolites undergo structural modifications, mainly in the liver, before entering the bloodstream [55–58].

Although initially considered indigestible due to their linkage to sugars, flavonoids undergo hydrolysis, allowing aglycone portion to penetrate the intestinal epithelial cells. After ingestion, flavonoid metabolites appear in the plasma after being processed by phase II enzymes in the liver and small intestine. However, the hydrophilic nature of glycosylated flavonoids limits their passive diffusion across the small intestine, resulting in minimal absorption. The instability of tea flavonoids in the colon may contribute to their poor bioavailability and absorption [59].

4. Approaches for enhancing the bioavailability of polyphenols

To address the challenge of limited bioavailability of polyphenols, nanotechnology, in particular nano-based carriers, has emerged as a promising avenue for enhancing the absorption of polyphenols. The minute size of nanoparticles allows them to traverse small capillaries and cells, facilitating maximal polyphenol accumulation and sustained release [60]. Investigations are being conducted into the use of lipid-based materials, dendrimers, and polymeric nanoparticles. Polymeric nanoparticles are favored due to their properties such as biodegradability and surface modifiability (**Figure 2**) [61].

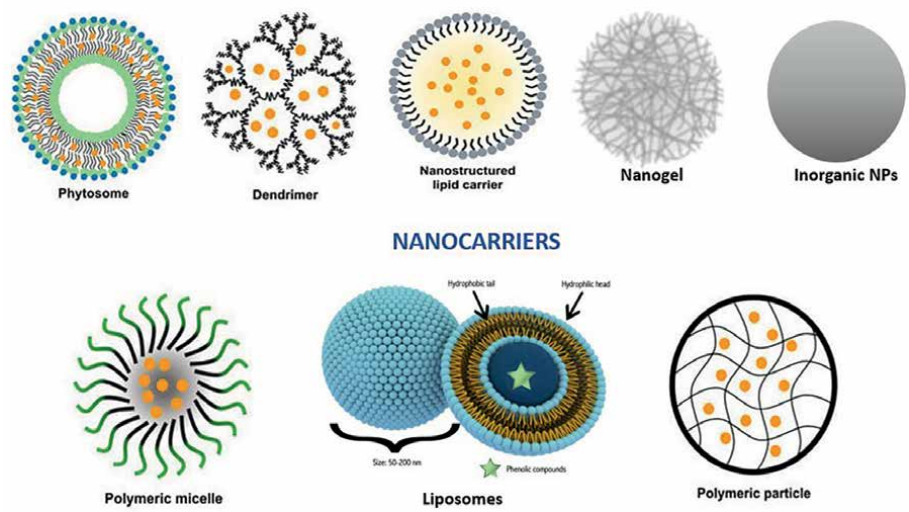

Figure 2.
Different type of nanocarriers used for polyphenols delivery.

Polyphenols can be protected by various nanocarriers, including polymeric nanoparticles, micelles, cyclodextrins, and gelatin [62]. Protein-based nanoparticles and chitosan are promising options for polyphenol nanocarriers [63].

A variety of nanoparticles have been employed to encapsulate and enhance the bioavailability of hydrophobic polyphenols. These include both organic and inorganic variants. Organic nanoparticles are derived from proteins, carbohydrates, and protein-polysaccharide complexes, while inorganic nanoparticles are primarily composed of gold, silver, and silica [64]. Polyphenols, including curcumin, rutin, and catechin, can be encapsulated using techniques such as nanoencapsulation and ionic gelation to enhance their bioavailability and stability [65, 66]. Studies on liposomal encapsulation and nanoencapsulation have highlighted the beneficial effects of polyphenols [67]. Nanoencapsulation has been shown to be a promising method for delivering various hydrophobic polyphenols *in vivo* [68].

There are a number of nanoscale delivery methods for polyphenols, including biopolymer-based nanoencapsulation, natural nanocarriers, and specialized device-based techniques. The use of nanoscale materials and structures allows researchers to overcome the barriers of absorption, distribution, metabolism, and excretion that often limit the bioavailability of compounds.

4.1 Nanoencapsulation

The use of nanotechnology to improve the bioavailability of polyphenols is an important area of research in the field of bioactive compounds. Nanoencapsulation, a leading technique in nanotechnology, is gaining popularity for its ability to enhance the protection and absorption of phenolics. By reducing particle size to the nanoscale, nanoencapsulation increases the surface-to-volume ratio, which improves bioavailability [69]. For instance, gelatin nanoparticles act as protective barriers for core polyphenols, such as resveratrol, shielding them from the harsh gastric environment and aiding in their transportation to the intestinal sites for efficient absorption [70]. Encapsulation systems, such as bovine serum albumin, can function as controlled release mechanisms, increasing the bioavailability of polyphenols like rutin while reducing their excretion rates [70]. The encapsulation of epigallocatechin gallate (EGCG) into chitosan nanoparticles (CSNPs) has been demonstrated to significantly inhibit the proliferation of human melanoma cells in both laboratory and animal studies [71]. Chitosan (CS), which is derived from crustacean shells, is commonly used in biomedicine due to its exceptional physicochemical and biological properties [72]. Additionally, CSNPs enhance the exposure of green tea extract to plasma by improving intestinal stability [73]. Consequently, nanoencapsulation represents a promising system for the efficient transport and release of phenolic compounds to target tissues. The encapsulation approach offers numerous benefits for enhancing the bioavailability of various polyphenols.

4.2 Lipid nanoparticles

Lipid nanoparticles (solid lipid nanoparticles, SLNs, and nanostructured lipids carriers, NLCs) are nano-sized (100–400 nm) colloidal lipid particles consisting of solid and surfactants with or without lipids. SLN and NLC were selected due to their numerous advantages, including high loading capacity, increased stability, controlled drug release, enhanced bioavailability, and biocompatibility for delivering natural compounds to the brain [74, 75].

The morphological structure of SLNs and NLCs resembles that of a lipid bilayer in a membrane, with surfactants on the outside and lipids in the center of the matrix. Based on its structure, NLCs can encapsulate both hydrophobic and hydrophilic drugs internally and externally, respectively. SLNs have the potential to improve the bioavailability of antioxidant nutraceuticals by increasing their solubility and permeability [76, 77].

The utilization of SLN and NLC in oral administration notably improved the bioavailability of curcumin extract [78]. *In vivo* studies demonstrated that SLNs loaded with resveratrol achieved a higher maximum plasma concentration compared to the physical mixture. Similarly, SLNs loaded with epigallocatechin gallate (EGCG) exhibited significantly higher maximum plasma concentration levels compared to free-form EGCG. Additionally, NLCs exhibited an initial burst release followed by a controlled release, ultimately leading to enhanced drug release and absorption, resulting in efficacious therapeutic outcomes [78].

4.3 Nanoliposome

Liposomes, small vesicles consisting of a bilipid layer enclosing an aqueous core, allow the encapsulation of both hydrophilic and lipophilic materials [79]. This encapsulation mechanism protects molecules from degradation and systemic dilution. Nanoliposomes not only encapsulate hydrophilic and hydrophobic substances, enhancing the solubility and utilization of polyphenols, but also control the release rate of these components, ensuring stability and efficacy [80]. In comparison with other nano-delivery systems, nanoliposomes have advantages such as ease of degradation, reduced immunity, reduced toxicity, and enhanced activity.

The encapsulation of epigallocatechin gallate (EGCG) in nanoliposomes, which are known for their stability under acidic conditions, followed by combination with alginate and chitosan particles, demonstrated high encapsulation efficiency (>97%) and a slow-release effect, effectively mitigating EGCG degradation [81]. This interaction enhances the bioaccessibility and/or bioavailability of polyphenols, exemplified by EGCG's ability to interact with lipid molecules, particularly in the lipid ester region. This results in the formation of stable and organized liposomes that prevent aggregation at higher catechin concentrations. As demonstrated by Tonnesen et al. [82], the incorporation of curcumin into liposomes resulted in a 20-fold increase in curcumin concentration in red blood cells when compared to the dilution of curcumin in Dimethylsulfoxide (DMSO). The improved solubility of quercetin in liposomes led to an increase in bioactivity, which was attributed to longer exposure of the cells to the active substance [83].

4.4 Phytosomes

Phytosomes are complexes of lipid molecules formed by binding plant extracts or their components to phospholipids, primarily phosphatidylcholines [84]. These complexes have been demonstrated to enhance bioavailability by providing an environment with increased lipophilicity. For instance, *in vitro* dissolution studies have shown that catechin-phospholipid complexes exhibit sustained release over 24 hours and superior antioxidant activity compared to free catechins at all tested doses [85]. Furthermore, the naringenin-phospholipid complex exhibited superior drug content and improved drug release in comparison with free naringenin during the *in vitro* dissolution studies conducted in distilled water [86].

4.5 Dendrimers

Dendrimers are branched polymer structures that have been extensively researched as effective drug carriers. Scientists are currently exploring new dendrimer-based formulations with the requisite properties for biomedical applications, including enhanced bioavailability, low toxicity, and high transfection profiles [87]. In order to combine the medical properties of caffeic acid with the drug delivery properties of dendrimers, a new class of polyphenolic dendrimers has been synthesized [88]. Grodzicka et al. [87] have synthesized carbosilane dendritic systems containing one or two caffeic acid units and ammonium groups on the surface to render them water-soluble. Their findings indicate that conjugation of polyphenols with cationic carbosilane dendrimers could be a promising approach to enhance the bioavailability of these powerful antioxidants.

To optimize the flavonoids bioavailability, Vergara-Jaque et al. [89] nanoencapsulated synthetic and natural variants, including quercetin, using G5-PAMAM dendrimers under both neutral and acidic conditions. The study revealed that the entrapment process was notably faster under acidic conditions than under neutral pH levels.

4.6 Polymeric micelles

Polymeric micelles, with a diameter ranging from 20 to 100 nm, are composed of amphiphilic polymer molecules. The use of polymeric micelles can help avoid unwanted effects [90]. The hydrophobic core of these micelles can encapsulate water-insoluble substances, while the hydrophilic corona protects the core, preventing removal by the reticuloendothelial system (RES), prolonging circulation time, and enabling interaction with blood components. Micelles have been observed to migrate from tumor vessel walls to cancer cells [91], as evidenced by the finding of Huan Li et al. [92], who conducted an *in vitro* gastrointestinal release test on micelles of curcumin and quercetin co-loaded. The test demonstrated that micelles exhibited pH-dependent release, releasing a small amount of polyphenol in simulated gastric fluid but presenting sustained release in the simulated intestinal fluid. The gastrointestinal-digested polyphenol-loaded micelles exhibited excellent antioxidant ability.

4.7 Nanogels

Nanogels are effective nanocarriers for delivering food-grade active substances to the gastrointestinal tract. This facilitates their conversion into particles that are crucial for the digestion and absorption processes [92, 93]. Jin et al. [94] developed a nanogel using soy protein and dextran self-assembly to deliver riboflavin effectively. Nanogels composed of carboxymethyl starch and chitosan hydrochloride, formed through chemical cross-linking, are effective carriers for bioactive compounds such as curcumin [95].

The self-assembly of natural proteins and polysaccharides into nanogels has garnered attention for their potential to deliver bioactive molecules. In this study, carboxymethyl starch-lysozyme nanogels (CMS-Ly NGs), synthesized through eco-friendly electrostatic self-assembly, were utilized to deliver epigallocatechin gallate (EGCG). The nanogels achieved an EGCG encapsulation rate of 80.0 ± 1.4% and maintained a stable particle size. Under simulated gastrointestinal conditions, CMS-Ly NGs with EGCG exhibited controlled release, which enhanced bioavailability. Furthermore, CMS-Ly NGs were able to encapsulate anthocyanins and

demonstrated slow-release properties during gastrointestinal digestion. These findings demonstrate the potential of protein and polysaccharide-based nanogels for delivery of bioactive compounds [96].

4.8 Inorganic nanoparticles

Due to their controllable size and shape, as well as their great specific surface area, inorganic nanoparticles have gained attention in enhancing the bioavailability of polyphenolic compounds. Gold and silver nanoparticles are the most widely used nanocarriers to enhance the bioavailability of polyphenols [97]. In addition, silica, titanium dioxide, and magnetic iron oxide nanoparticles have also been used in drug-delivery systems [98–100].

Several studies have shown that silver and gold nanoparticles have the potential to serve as nanocarriers to improve the bioavailability of curcumin [98, 101]. Additionally, research has demonstrated that EGCG-gold nanoparticles have greater anticancer efficacy than free EGCG and EGCG-gold nanoparticles synthesized through the citrate method [90]. Gold nanoparticles are excellent nanocarriers for tracking the absorption of polyphenols through imaging techniques due to their small size.

5. Nanotechnology challenges

Nanopolyphenols present several challenges regarding their biological properties, biocompatibility, and safety. In the field of nanomedicine, these challenges include limited understanding of nanomaterial interactions with tissues and cells, as well as uncertainties regarding their biological interactions within the body. Additionally, there is a need for specialized toxicological studies for nanopolyphenols and ensuring structural stability post *in vivo* administration. It is also important to address the accumulation of nanoparticles in target organs, tissues, and cells. These challenges have been previously highlighted studies [102]. Additionally, the high cost of raw materials required for nanopolyphenols synthesis presents challenges for scalability and manufacturing. To justify these expenses, nanomedicine products must exhibit significantly improved clinical therapeutic effects compared to conventional therapies [103].

Despite these challenges, the use of nanotechnology in designing and formulating nanomedicines derived from polyphenols is expanding in both nutraceutical and pharmaceutical markets. Nevertheless, comprehensive nanotoxicity studies remain insufficient, despite clinical trials indicating only mild adverse effects resulting from nano-polyphenol administration [104]. To address potential nanotoxicity challenges, it is necessary to have adequate screening platforms that can predict the toxicological behaviors of nano-formulated products. This will enable the implementation of safety parameters that meet international standards [105].

6. Conclusion and future perspectives

Despite the numerous challenges associated with polyphenol bioavailability, such as low solubility, susceptibility to degradation, and limited absorption, nanotechnology offers a promising approach and innovative solution to overcome these

obstacles. Techniques such as nanoencapsulation, lipid nanoparticles, nanoliposomes, phytosomes, dendrimers, polymeric micelles, and nanogels protect polyphenols from degradation in the gastrointestinal tract, enhance their transport to target sites, and improve their absorption and therapeutic efficacy.

However, the field of nanopolyphenols also presents several challenges that need to be addressed. These challenges include understanding the biological properties and interactions of nanomaterials, ensuring biocompatibility and safety, addressing potential nanotoxicity, and optimizing manufacturing processes to reduce costs and scale up production. However, comprehensive toxicological studies are needed to assess the potential risks associated with nanopolyphenols in order to fully exploit the therapeutic potential of polyphenols.

Overall, the integration of nanotechnology with polyphenol delivery systems holds great promise for revolutionizing the use of polyphenols in functional foods, nutraceuticals and pharmaceuticals. Continued research and development in this area is critical to unlocking the full potential of polyphenols in promoting human health and combating various diseases.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 6

Impact of Nanomaterials on Wine Quality: A Focus of Siliceous, Aluminosiliceous, and Carbon-Based Nanomaterials on the Phenolic Fraction of Wine

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Abstract

Nanomaterials represent reduced scale versions of conventional materials, their nanometric structures possessing totally different and unexpected properties in contrast to the same substance at the macroscopic level. Nanomaterials present crystalline structure and manifest high resistance at high temperatures and acidic pH. Due to these properties, nanomaterials have widespread applications in different areas, such as pharmaceutics and wine industry (the degradation or removal of pollutants, the immobilization or vectorization of yeast, the increasing content of bioactive compounds). Nowadays, consumer demanding is centralized on wines with interesting sensory profile and increased nutritional value. Phenolic compounds play pivotal roles in shaping the sensory attributes of wines. The integration of nanomaterials can contribute to augmenting the extraction of phenolic compounds, depending on the specific type of nanomaterial employed, its concentration, and the particular winemaking technology applied. This chapter is focused on the impact of siliceous and aluminosiliceous porous and carbon-based materials on the phenolic profile of wines. In accordance with the existing studies, phenolic profile of wines is selectively modified by nanomaterials, but a more-deep comprehension of the nuanced interaction between nanomaterials and phenolic compounds is anticipated, offering insights that may underpin innovative strategies aimed at enhancing the overall quality of wines.

Keywords: siliceous materials, aluminosiliceous porous materials, carbon-based nanomaterials, phenolic compounds, wine quality

1. Introduction

Nanoscience focuses on studying innovative materials and technologies applied at the nanoscale, typically with particle sizes ranging from 1 to 100 nm. Nanoparticles can be used in viticulture as fertilizers, pesticides, and fungicides in the management of diseases (anthracnose, downy mildew, and powdery mildew) and vine pests (flea beetles, bugs, thrips, red mites, fungi, hyacinths, and stem borers). Conventional fertilizers can be replaced by nano-fertilizers (metallic nanoparticles; metal oxide nanoparticles; nanocomposites from nitrogen, phosphorus, potassium; micronutrients; hydroxyapatite; double layered zinc-aluminum hydroxide; and zeolites,) which show high efficiency, having the ability to reduce the frequency of application to increase the availability of nutrients and stress tolerance toward crops [1]. Moreover, nanomaterials could contribute to the optimization of wine technologies to enhance stability and improve both structure and composition [2]. Nanoporous materials, characterized by their substantial adsorption capacity, are driven by high specific surface area, pore volume, nanopore size distribution, and precisely ordered structure and also exhibit notable selectivity. This selectivity arises from a narrow pore size distribution and specific interactions with pore walls. Additionally, their favorable adsorption kinetics, stemming from a regular pore structure, dimensional characteristics, and pore sizes, ensure stability, durability, and the reversibility of adsorption and desorption processes. Notably, these materials demonstrate commendable mechanical properties in resisting abrasion and compression [3].

Nanomaterials have been used in wine quality analysis equipment. This allowed for improving the performance of the instruments by simplifying the working methodology, reducing working time, using a small sample volume, and reducing or even eliminating the pre-processing stage of samples. Some nanoparticles have been studied as part of detectors to analyze the total polyphenol content of must and wine (gold, silver, and zinc dioxide nanoparticles or multi-walled carbon nanotubes). Nanoparticles with magnetic properties have been used to create high sensitivity detection methods to analyze the content of ocratoxin A and histamine in beverages. In consequence, analysis methodologies using gold nanoparticle biosensors have been developed to allow the detection of low concentrations of ochratoxin A (up to 0.068 ng/mL). Also, electrodes functionalized with a nanostructured sensing surface that includes various nanoparticles can not only be used to detect polyphenols, sulfur dioxide, and glycerol but also some unwanted yeasts, such as *Brettanomyces bruxellensis* [4, 5].

In the wine industry, the treatment of different nanomaterials has been studied to reduce the concentration of various pollutants and the oxidative processes, thus inducing better stability and safety [6–10]. Phenolic compounds are natural components found in grapes, and while they contribute to the flavor and color of wine, excessive amounts can negatively impact its quality. Therefore, the removal of excessive phenolic compounds is a desirable process in winemaking. The focus of this chapter is that of reducing the content of phenolic compounds responsible for oxidative processes in wine, thus ensuring better stability and improved sensory characteristics. These treatments were also carried out in order to follow their selectivity in the retention of different phenolic compounds from the wine.

2. Key-phenolic compounds in wine

Wine represents a complex system characterized by a diverse array of phenolic compounds, playing a pivotal role in determining the wine's quality, its sensory

attributes, and imparting valuable antioxidant properties [11]. Among the phenolic compounds derived from grapes, anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, phenolic acids, and stilbenes can be mentioned. These molecules come from different parts of the grape clusters, being extracted during winemaking. In grapes, phenolic acids are primarily present as glycosidic compounds, which can undergo decomposition through acid hydrolysis or as esters (tannins, gallic, and ellagic acids), which are released through alkaline hydrolysis. Free forms, particularly prevalent in red wine, result from the hydrolysis of phenolic acid combinations and the breakdown of more complex molecules (anthocyanins). Phenolic acids, although colorless in alcoholic solutions, may turn yellow after oxidation reactions (gallic acid, protocatechuic acid, caffeic acid, ferulic acid, and so on.) [12]. The main phenolic compounds existent in wines are presented in **Figure 1**.

Despite lacking distinctive taste or odor, these compounds serve as precursors for certain volatile phenolic compounds that may emerge due to interactions with specific microorganisms in wine. When the wines are aged in new oak barrels, the wood used in the manufacture of the barrels leads to the formation of new compounds (ethyl phenol, vinyl phenol, guaiacol, methyl guaiacol, vinyl guaiacol, propyl guaiacol, etc.) with a smoky and burned smell. The phenolic profile of grapes and wine is strongly affected by different factors such as varietal variability, cultivation technology, climate and soil conditions, pathogen attacks, winemaking practices, etc. [12]. For example, the application of mechanized harvesting practices can induce a substantial increase in the content of polyphenols in wine. The concentration of phenolic compounds transferred from the raw material can result in concentrations of up to approximately 4 g/L gallic acid in red wines and 350-500 mg/L in white wine samples [11]. **Table 1** presents the medium levels of phenolic compounds in wines.

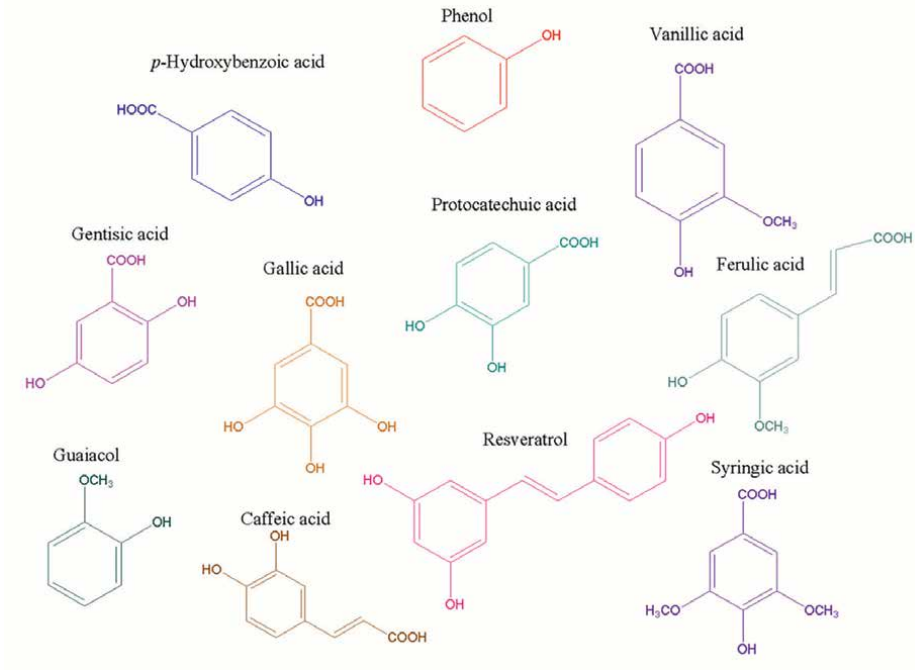

Figure 1.
Key-phenolic compounds from wine.

Compound name	White wines (mg/L)	Red wines (mg/L)
Benzoic acids	1–5	50–100
Cinnamic acids	50–200	50–200
Flavonols	Traces	15
Anthocyanin	0	20–50
Monomeric flavanols	Traces	150–200
Procyanidins	<100	1500–5000

Table 1.
Phenolic compound levels in white and red wines [13].

Hydroxycinnamic acids, including caftaric, coutaric, and fertaric, often show widely varying concentrations, their level usually being below the detection threshold. When combined with other wine components, these non-flavodoids significantly impact the organoleptic perception, particularly in relation to the alcoholic strength. For example, caftaric acid has been noted to enhance bitterness perception, while p-coumaric acid may imprint nuances of myrrh and cinnamon, etc. Moreover, these compounds have bactericidal, choleretic, and diuretic properties in the human body [12].

Beyond phenolic acids, musts and wines contain other crucial compounds for assessing their organoleptic quality, known as stilbenes. The predominant stilbenes in wines are resveratrol, tyrosol, methoxyrosol, and tryptofol. For example, the production of tyrosol by yeast metabolism can contribute to the imprinting of the bitter taste, particularly notable in sparkling wines. The level of this compound considerably rises during the second fermentation in the bottle. Even in white wines, tyrosol can impart bitterness at levels of 25 mg/L [11].

Volatile phenols, derived from phenol, play a major role in defining the aromatic profile of wine [14]. About 20 such compounds have been identified in wine, some of them being presented in **Table 2**.

Tanning substances, primarily synthesized by plants, can be categorized into hydrolyzable and condensed types. Hydrolyzable tannins, based on nonflavonoid phenols, are found as esters and are susceptible to degradation or hydrolysis. Condensed tannins (procyanidins) cannot be easily broken down by hydrolysis. Wine tannins consist of polymers of leucoanthocyanidins and catechins. White wines contain small amounts of catechins (3-flavanol) and leucoanthocyanins (3,4-flavandiol), contributing to the wine's structure and body [11]. Tannins form blue-colored complexes upon reaction with Fe^{3+} and interact with proteins, imprinting astringency. They can originate from all the solid grape parts. On average, 58.5% of condensed tannins are found in seeds, 21% in stems, 16.5% in leaves, and the remaining 4% in berry skins across different grape varieties. During aging, tannin concentrations in wines decrease significantly as a result of oxidation and protein precipitation [17]. These substances are of particular importance in defining the quality of wines, their stability, and evolution (they have important antioxidant action). From an organoleptic point of view, tannins give hardness, astringency, as well as bitter and sour notes, proportional to their concentration [14]. Bitterness perception is mediated by taste buds on the tongue, and various phenolic compounds activate distinct combinations of bitter taste receptors. Astringency, on the other hand, involves dryness and puckering, and it is linked to the interaction of tannins with salivary proteins, influenced by factors such as tannin structure, wine matrix composition, and saliva characteristics. Seed proanthocyanidins were perceived as more

Compound name	Detection limit (μg/L)	Origin	Aroma descriptors	References
Phenol	30	Lignin degradation	Chemical	[15]
Guaiacol	23		Smoke, sweet	[11]
4-Methyl guaiacol	21		Smoke, ash	[16]
Syringol	57		Smoke, drugs	[11]
Eugenol	6		Cloves, spicy note	[16]
Vanillin	200		Vanilla	[11]
M-cresol	20		Leather	[16]
4-Ehylphenol	440	*Brattanomyces spp.*	Leather, manure	[11]
4-Vinylphenol	180		Medicinal, phenolic, tobacco	[11]
4-Ethylguaiacol	33		Spicy, cloves	[11]
4-Vinyl guaiacol	40	*Saccharomyces spp.*	Smoke, phenolic	[16]

Table 2.
Key volatile phenols in wine.

astringent than skin proanthocyanidins, and specific subunits of tannins impact bitterness sensation. The amount of saliva also affects the perception of astringency with a linear correlation found between protein concentration and tannin-binding affinity. Salivary proteins, including the PRP family, α-amylase, statherin, histatins, and mucins, exhibit diverse abilities to interact with tannins, with some proteins specifically binding to astringents [12].

The coloring substances in wine are mainly represented by anthocyanins (in red wines) and flavones (in white wines). Anthocyanins exist as glycosides and are often 0linked or acylated with acetic, caffeic, or p-coumaric acids. Their color and intensity are not only given by the number of hydroxyl groups that bind to the benzene nucleus but also by the pH of the medium. They are primarily located in the skin and occasionally in the pulp. They also exist in significant quantities in the leaves toward the end of the vegetation period. Structurally, anthocyanins feature a flavylium cation structure, consisting of two benzene rings connected by a positively charged unsaturated oxygen heterocycle derived from a 2-phenyl-benzopyrylium nucleus. These molecules are more stable when found in the glycosidic form (anthocyanins) compared to the aglycone form (anthocyanidins). The color of these pigments is determined by environmental conditions (pH, SO_2) and molecular structure. On the one hand, the substitution at the lateral benzene nucleus leads to a bathochromic change of the wavelength with maximum absorption (to violet). Moreover, glucose fixation and acylation change the color to orange. Predominantly located in the cells of seed skin, these pigment molecules form a concentration gradient from the interior to the exterior. In the presence of other polyphenols (phenolic acids, flavonoids, etc.), these molecules exist in solution, influencing their color. These factors contribute to the diverse range of colors observed in red grape varieties. While all grape varieties share a fundamental structure for anthocyanidin, with minimal compositional variations, the malvidin molecule is dominant across all grape varieties. Malvidin-monoglucoside

plays a crucial role in determining the color of red wines, with its concentration varying based on the specific grape variety [18].

In white wines, flavones are the main responsible for their yellow-brown color. From a chemical point of view, the structure of these compounds is similar to that of anthocyanins. The main flavonic compounds that can be separated in wine are represented by quercitin, kaempferol, and myricetin [19].

2.1 Phenolic oxidation in wines

Changes in the phenolic profile can occur due to the participation of these compounds in various chemical reactions (copigmentation, cycloaddition, polymerization, and oxidation). Copigmentation (additional colorless flavonoids known as cofactors or "copigments" enhance the pigmentation caused by anthocyanidins) generally gives a purple tint to wine. These reactions begin immediately after the grapes are crushed and continue during the fermentation and aging period, helping to define the sensory properties of the wines, in particular color and astringency. Iron plays a role in this process by reducing oxygen to form the hydroperoxyl radical (often known as hydrogen superoxide, which is a protonated type of superoxide with the chemical formula HOO• with a crucial role in cell biology). Phenolics, particularly those with specific hydroxyl group configurations, exhibit high reactivity toward these radicals, forming stabilized semiquinone radicals (**Figure 2**). Examples of phenolics prone

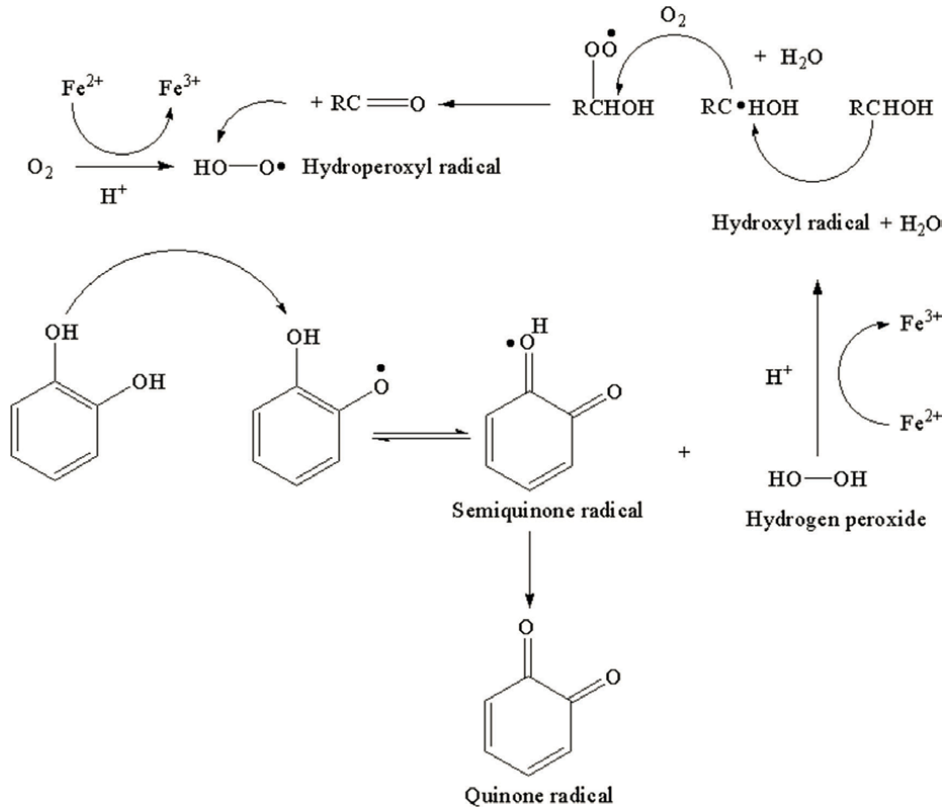

Figure 2.
Oxidation of phenolic compound [20].

to oxidation include caffeic acid, catechin, and quercetin. Monophenols and non-catechol phenolics are less susceptible to oxidation. The primary wine anthocyanin, malvidin-3-glucoside, shows a good resistance to oxidation processes. The reactivity of oligomeric and polymeric phenolics, such as procyanidins and condensed tannins, is similar to monomeric vicinal dihydroxy phenolics in the presence of reactive oxygen species. Overall, understanding the oxidation dynamics of these phenolic compounds is crucial for preserving the quality of wines [20].

Understanding the relationship between the quality of a given wine and its phenolic composition remains one of the major challenges in oenological research [21].

3. Wine conditioning and stabilization using nanostructured materials

Preserving the distinctive qualities, taste, color, and aroma is a crucial aspect for all types of wines, including red, rosé, and white varieties. The inherent oxidative processes in wine can lead to taste deterioration, diminished vibrancy, and the development of oxidation tones, ultimately compromising the wine's quality. To address this challenge, various technological methods are employed throughout the winemaking process. This includes careful selection of temperature regimes and the utilization of specific oenological products designed for clarification and stabilization. The wine clarification process is dedicated to eliminating suspended and colloidal particles that contribute to turbidity. Simultaneously, it aims to remove unstable proteins and other macromolecules that might undergo denaturation or aggregation, resulting in a cloudy appearance post-bottling. Stabilization is achieved through adsorption processes, employing substances such as bentonite, chitosan, fish glue, and albumin, and polymeric compounds such as PVP (Polyvinylpyrrolidone), PAA (Polyacrylic acid), and PEO (Polyethylene oxide). These materials effectively target polyphenols, proteins, and crystalline compounds, ensuring stability. Additionally, membrane filtration is employed to contribute to stabilization [22].

3.1 Bentonite treatment

Bentonites are hydrated aluminosilicates, mainly composed of montmorillonite that exhibit distinctive physicochemical properties influenced by their geographical origin. While German or North African bentonite is rich in Ca^{2+} ions, the variety from the United States contains Na^{+} ions and is widely recognized as highly effective in wine treatment. The colloidal properties of bentonite stem from its layered structure, enabling significant swelling in aqueous environments, boasting a large adsorption surface, and carrying a substantial negative charge. The crystal-chemical structure of clay minerals, specifically montmorillonite, involves layered arrangements of tetrahedral and octahedral layers, forming 1:1 or 2:1 type packages. Montmorillonite, the primary component of bentonite, is not abundantly found in its pure state in nature. However, its cation exchange capacity can range from 70 to 100 meq/100 g of clay, with specific surface areas up to 120 m^2/g and effective pore sizes characteristic of montmorillonite. Recommended for wine treatment, bentonites undergo activation with sulfuric acid or alkaline salts. Their high ionic exchange capacity allows loading with H^{+}, Na^{+}, or Ca^{2+} ions, resulting in acidic, calcium, or sodium forms of bentonite. The sodium form is particularly favored, exhibiting extensive swelling in wine, high adsorption capacity for proteins, and maintaining a stable colloidal character. This treatment effectively removes natural proteins, resulting in a clear liquid and

protecting the wine from copper case. Bentonite treatment stands out as the prevalent method for removing excess proteins, especially in red wines with elevated concentrations of colored colloids generated through heating or harsh mechanical treatments applied to grapes. While effective in stabilizing wine, this treatment comes with the trade-off of a noticeable loss of color [23].

3.2 Ion-exchange materials

The treatment with ion-exchange materials involves the use of insoluble polymeric resins (formed by styrene and divinyl benzene) activated with various functional groups in ion-exchange reactions (sulfonic group or carboxyl group for cation exchange and the quaternary ammonium ion or the salt of a tertiary amine for the anion exchange) [24]. The resin presents a three-dimensional (0.3-1.2 mm diameter) and porous matrix that supports the ion-exchanging groups and consists of a synthetic resin that is obtained by condensation and polymerization. The appearance of the resins can be observed in **Figure 3**. Ion-exchange resins are usually activated by treatment with an acid of mineral origin (sulfuric acid), incorporating H^+ ions. The activated resin exchanges its H^+ ions with cations (e.g., K^+, Ca^{2+}, and among others) present in grape must and wines. The exchange is stoichiometric and generates a reduction in the pH value and an increase in acidity, organic acids being released. Cationic resins follow the laws of affinity. Thus, higher valence cations are preferred to be exchanged over lower valence ones ($Al^{3+} > Ca^{2+} > K^+$). Also, the divalent cations (Mg^{2+} and Ca^{2+}) of the must and wines are fixed in the cationic resin in preference to the monovalent ions (like Na^+ and K^+). However, if two cations have the same valency, the preference is for the higher atomic number [26].

3.3 Carbon-based materials

Carbon nanotubes are cylindrical nanostructures with exceptional thermal and electrical conductivity and high mechanical strength and rigidity. One advantage of using carbon nanotubes is that they can be regenerated and reused, making process economically viable. Consistent with Mamvura et al. [27], carbon-based nanomaterials can be an effective alternative as artificial flocculants to improve the flocculation capacity of brewer's yeast. Used as filter membranes, carbon nanotubes remove multiple components of heavy hydrocarbons from oil, while in water, they remove bacteria, such as *Escherichia coli* and 25 nm nano-sized polyviruses [6, 28].

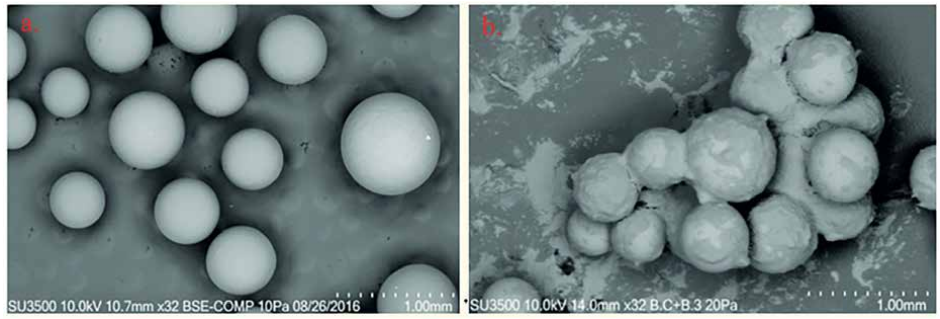

Figure 3.
Examples of SEM images with the resin before (a) and after (b) Cd^{2+} adsorption [25].

Considered the thinnest material in existence [29], graphene is the two-dimensional version of graphite, consisting of a two-dimensional arrangement of carbon atoms arranged in a hexagonal lattice. Also, this material is the best-known conductor of electricity and heat. Some typical appearances of carbon-based nanomaterials can be analyzed in **Figure 4**. Graphene and reduced graphene oxide, which is graphene oxide that has been reductively processed by various methods to reduce its oxygen content, has been used to fabricate biosystems, consisting of nucleic acids, peptides, proteins, and enzymes [30], active adsorbent materials that remove heavy metal ions, pesticides, and natural dyes from water [31, 32]. Colibaba et al. [33] obtained an important increase of proline and threonine in wines treated with graphene oxide, while phenylalanine, arginine, and tyrosine level were decreased. Also, carbon nanotubes generated the diminishing of shikimic and fumaric acids.

3.4 Microporous zeolitic materials

Zeolites, whether of natural or synthetic provenance, are crystalline aluminosilicates hydrated with alkaline and alkaline metals such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+}, with pore diameters less than 2 nm (**Figure 5**). These materials are considered safe for human consumption, being used not only in water and soil decontamination, in agriculture (in soil corrections and carriers for fertilizer) but also in food and beverage processing. In the latter context, zeolites find application in tasks such as tartrate and protein stabilization, prevention of light-struck taste, removal of off-flavors and metals, waste management, and cell immobilization. Moreover, zeolites are used as catalysts, detergents, adsorbents, molecular sieves, and ion exchangers in diverse chemical processes. The foundational structure of zeolites is

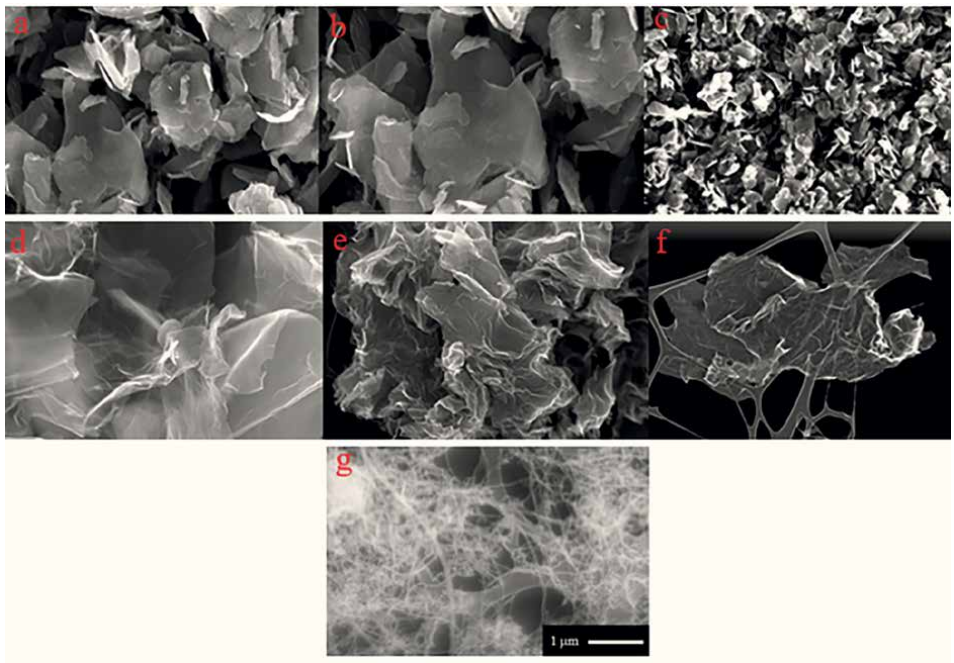

Figure 4.
Typical SEM images of graphene nanopowder (a), graphene oxide (b), and carbon nanotube powder (c) [29].

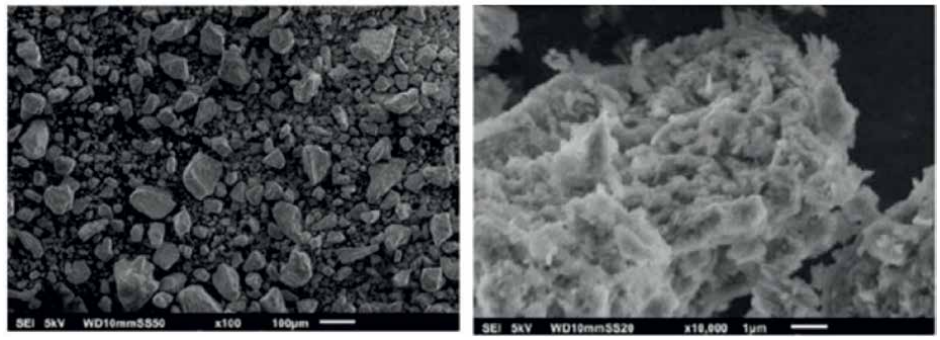

Figure 5.
Examples of SEM images for zeolite [34].

three dimensional, arising from the interconnection of tetrahedral TO_4 units (SiO_4 and AlO_4) through oxygen bridges. Within standard conditions, the cavities and/or channels are occupied by metallic cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}) or organic cations required to compensate for the excess negative charge of the tetrahedra (AlO_4), a consequence of the presence of Al(III) and water molecules. The mobility determined by both cations and water molecules facilitates reversible ionic exchange and dehydration processes. Among the most used zeolites in the oenological processes are Charazite, Clinoptilolite, Edingtonite, Faujasite, Mordenite, Phillipsite, and Linde Type A [35].

3.5 Mesoporous materials

Mesoporous materials are obtained by combining specific proportions of inorganic compounds (sodium silicate solution, aerosol) or organometallic compounds (such as alkylated silica) with the organic molecules of surfactant substances acting as structure-directing agents (templates). These materials usually present cylindrical pores with 2-50 nm diameter and large surface area (700-1500 m^2/g). Silicon-based mesoporous materials can be formed by pure (MCM, SBA, HMS) or modified silicates (which include transition metal oxides and nonmetallic oxides). Mesoporous siliceous oxide has multiple industrial applications, including adsorption, ion exchange, catalysis, photocatalysis, chemical and electrochemical sensors, permselective membranes, electronic relays, zeolitic batteries, rapid ion conductors, semiconductors, thin films, materials for data and image storage, molecular-sized electronic, optical, and magnetic devices, and controlled drug release systems, among others [36].

Mesoporous silica nanomaterials (MSN) are often used in the pharmaceutical field due to their association with numerous benefits to human health. The group of M41S-type mesoporous materials comprises three other types, namely MCM-41 (hexagonal), MCM-48 (cubic), and MCM-50 (lamellar). Of these, the M41S variant has a pore size between 2 and 20 nm and a specific surface area of up to 1000 m^2/g. In addition, this type of material has distinct adsorption properties associated with its pore volume (around 0.9 cm^3/g) [10]. Various mesoporous silica materials, including MCM (Mobil Composition of Matter), SBA (Santa Barbara Amorphous), FDU (Fudan University), and KIT (Korean Institute of Science and Technology), have been successfully synthesized using different templating methods. KIT-6 silica, in particular, exhibits a bicontinuous cubic structure with Ia3d symmetry and features interpenetrating cylindrical pores, making it well-suited for serving as a hard

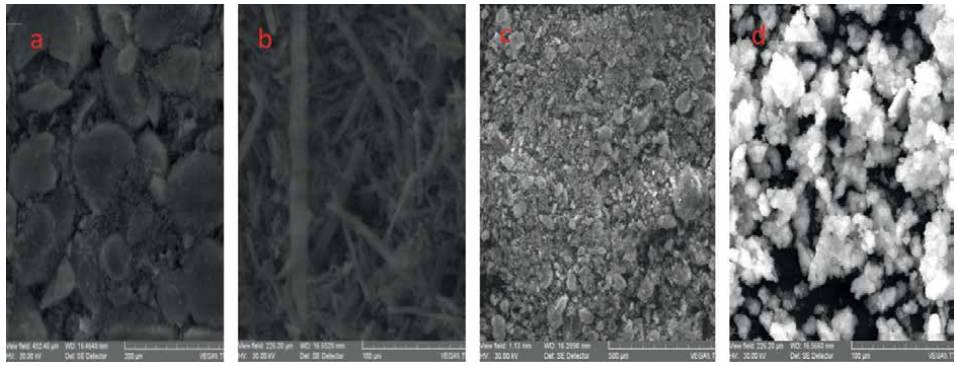

Figure 6.
SEM images of calcinated samples of Al-MCM-41 (a), SBA-15 (b), MCM-41 (c), and KIT-6 (d) (original).

template and catalyst support. The synthesis process allows for the precise control of mesoporous silica's pore size by adjusting factors such as reaction temperature, surfactant type or concentration, and swelling agent concentration. This control over pore size is crucial in adapting KIT-6 for the production of specific target materials with enhanced properties [37].

SBA-15 demonstrates compelling textural characteristics, notably large specific surface areas exceeding 1000 m^2/g, uniformly sized pores ranging from 4 to 30 nm, substantial framework walls, small crystallite size of primary particles, and complementary textural porosity. The utilization of SBA-15 as a support offers additional benefits, including a high surface-to-volume ratio, versatile framework configurations, and elevated thermal stability [38].

These materials are efficient as potential supports in catalysis or for the adsorption of molecules with large molecular sizes and can be synthesized by various methods such as sol-gel processing, template-assisted techniques, microwave-assisted techniques, and chemical etching techniques [36]. **Figure 6** illustrates some SEM images of different types of mesoporous materials.

Some of these materials were used by Dumitriu et al. [8] in the production of some wine samples. The authors presented that MCM-41 and SBA-15 can reduce significantly turbidity units of wine, while KIT-6 had low effects on the final protein level.

The focus on developing and investigating novel polymeric materials and adsorbents, primarily derived from bio-renewable plant sources, provides insights into their potential targeted application for producing high-quality wines.

4. Synthesis of siliceous, aluminosiliceous, and carbon-based nanostructured materials for beverage manufacturing usage

Carbon nanotubes exemplify innovative nanostructures derived from chemical synthesis approaches. Despite their simple chemical composition and atomic bonding configuration, nanotubes showcase remarkable diversity and richness among nanomaterials in terms of structures and structure-property relationships. Metallic and semiconducting nanotubes have been identified across materials synthesized through arc discharge, laser ablation, and chemical vapor deposition methods [39]. Elemental carbon in sp^2 hybridization can create various remarkable structures. In addition to the well-known graphite, carbon can construct closed and open cages with

a honeycomb atomic arrangement. Nanotubes consist of up to several tens of graphitic shells, known as multi-walled carbon nanotubes, with adjacent shell separation of 0.34 nm, diameters of 1 nm, and a high length-to-diameter ratio. Generally, carbon nanotubes have diameters ranging from less than 1 nm up to 50 nm as a group. While their lengths are typically several microns, recent advancements have extended the nanotubes to much longer lengths, measured in centimeters. The choice of carbon nanotubes for phenolic compound adsorption should be guided by experimental testing and optimization. Factors, such as the type of phenolic compounds, concentration, and desired properties of the final product, should be considered in the selection process. The literature mentions many strategies for manufacturing large amounts of carbon nanotubes: arc discharge, laser ablation, chemical vapor deposition, silane solution method, and flame synthesis method. The electric arc discharge technique uses high temperatures above 1700°C between water-cooled graphite electrodes in a helium-filled chamber at subatmospheric pressure. Different gases, such as helium, methane, and hydrogen, lead to variations in the final structure. The existing papers indicate that organic molecular atmospheres contribute to higher multi-walled carbon nanotube yields, and the pressure also plays a role. Maintaining the desired distance between electrodes during the growth process, typically between 1 and 4 mm, is crucial for achieving a high yield and stable arc discharge growth process. The electrode feed is essential to ensure a constant gap and facilitate efficient multi-walled carbon nanotube synthesis [40].

Activated carbon is a versatile and highly porous material with a wide range of applications, including water purification, air filtration, gas adsorption, and more. The synthesis of activated carbon involves the carbonization of carbon-rich raw materials followed by activation processes to enhance its porosity. Common raw materials include coconut shells, wood, peat, coal, sawdust, and agricultural residues. The selected raw material should have a high carbon content to ensure a more effective activation process. The raw material undergoes carbonization in the absence of air or in a controlled environment with limited oxygen. This process involves heating the material to high temperatures (typically 600-900°C) to remove volatile components and convert the material into a carbon-rich structure. Activation is a crucial step that introduces porosity to the carbon structure and involves impregnating the carbonized material with chemical agents, such as potassium hydroxide or phosphoric acid. The impregnated material is then heated to activate and create pores. To ensure the physical activation of the carbon, high temperatures, and gases are needed, such as steam or carbon dioxide. The temperature during the activation process influences the properties of the activated carbon. Higher temperatures generally result in increased porosity. The activated carbon is cooled and then thoroughly washed to remove any residual impurities or chemicals used during activation. The final activated carbon product is dried to remove excess water. The activated carbon may undergo grinding and sizing processes to achieve the desired particle size for specific applications. Activated carbon is characterized based on parameters such as surface area, pore size distribution, and adsorption capacity [41].

Jiříčková et al. [42] proposes a method to obtain graphene oxide. In this, a mixture of phosphoric acid and sulfuric acid in a ratio of 1:9 is mixed with potassium permanganate and graphite in a ratio of 6:1, all in an ice bath. The mixture is heated to 50°C and stirred for about 12 hours. After cooling, the solution is poured onto ice and 30% H_2O_2 is added to remove excess potassium permanganate. More modern approaches to oxidizing graphite to prepare graphene oxide include using potassium chromate with perchloric or nitric acid. Alternatively, potassium ferrate, considered to be less toxic

in sulfuric acid, has been proposed. Oxidation of graphite can also occur in water with H_2O_2 at 50°C by Fe(VI) or at 110°C by benzoyl peroxide. It is worth noting that chemically prepared graphene oxide often exhibits a highly damaged structure due to harsh acidic conditions and the presence of impurities, making it less suitable for electronic applications. While chemical methods, particularly the chlorate and permanganate processes, produce graphene oxide with suboptimal electrical properties, ongoing research aims to improve these methods. Electrochemical production of graphene oxide is considered more environmentally friendly than chemical methods due to the reuse of electrolytes and minimal washing of utensils. The use of aqueous electrolytes and the absence of oxidizing agents contribute to the superior quality of electrochemical graphene oxide compared to standard procedures. Also, the use of biological systems, such as *Acidithiobacillus ferrooxidans* or *Pseudomonas,* has been explored to oxidize graphitic materials in an environmentally friendly manner. Studies have shown that, however, after microbial cultivation, graphite oxidation may not be homogeneous.

The procedure for synthesizing mesoporous silica material SBA-15 is proposed by Zhao et al. [43] and by Luchian et al. [10]. The hydrothermal synthesis of the material can be achieved using a reaction system with the following molar composition: $1SiO_2$: 0.017 P123: 5.87 HCl: 194 H_2O.

The process of obtaining solid SBA-15 powder consists of dissolving 4 g of P123 in 150 mL of 2 M acidic HCl solution. Drops of tetraethylorthosilicate are added under continuous stirring (9.6 mL). The mixed solution is kept at 45°C for 8 h, and finally, the sol–gel suspension is heated to 80°C for 5 h in a conventional oven. Next, the white solid is filtered, which is washed several times with deionized water and dried at room temperature and finally by calcination at 550°C for 6 h (heating rate of 10 C/min).

The synthesis of mesoporous KIT-6 silica can be achieved following the proposal of Xiaoying et al. [44]. For this, 5 g of Pluronic® P123 (amphiphilic nonionic triblock copolymer) is dissolved in 180 g of distilled water and 9.9 g of HCl solution (35%) under vigorous stirring at 35°C. After complete dissolution, add 5 g of n-butanol (99.4%). After additional stirring for 1 h, immediately add 10.75 g of tetraethylorthosilicate. The mixture was stirred at 35°C for 24 h and then transferred to an autoclave, which, in turn, was sealed and kept at 100°C for 24 hours. The resulting solid product was filtered and dried at 100°C overnight. After a brief ethanol/HCl wash, dry the final sample at 70°C and calcine at 550°C for 6 hours in air.

For the synthesis of the Al-MCM-41 mesoporous material, the method presented by Stein & Holland [45] is proposed. Thus, 22.3 mL of tetraethylorthosilicate was mixed with 0.68 g of aluminum isopropoxide. The resulting solution is stirred for 30 minutes at 250 rpm, after which tetraethylammonium hydroxide solution (10% water) is added with continuous stirring for another 30 min, at a speed of 250 rpm until the gel is formed (pH = 11). A total of 7.2 g (0.2 mol) of cetyltrimethylammonium bromide (30 mL/h) was added dropwise. The gel becomes a suspension. After additional stirring for 1 h, transfer to a Teflon steel autoclave and heat at 150°C for 48 h. After cooling, the sample is recovered by filtration. The obtained solid is washed with distilled water and ethanol, then air-dried at 70°C for 1 hour, and finally calcined at 540°C for 6 hours.

5. Impact of siliceous, aluminosiliceous, and carbon-based nanomaterials on wine phenolic profile

As research in nanomaterial applications continues to evolve, exploring the synergy between carbon nanotubes and winemaking processes opens new opportunities for

innovation and quality improvement in the wine industry. The adsorption process involves the attachment of phenolic compounds to the surface of carbon nanotubes through Van der Waals forces, π-π interactions, and hydrogen bonding. Several papers published by our team presented the impact of carbon-based materials on wine phenolic profile (**Figure 7**). Codreanu et al. [6] investigated the impact of carbon-based materials on the phenolic profile of wines from Romania. Different nanomaterials (carbon nanotubes, graphene, and oxide graphene) have been introduced in both the pre- and post-alcoholic fermentation stages of Cabernet Sauvignon wines (**Figures 8** and **9**). The authors obtained an important decrease in phenolic content when carbon-based materials were used. Also, the results were influenced by the moment of application. So, when the materials are applied before alcoholic fermentation, the total content of phenolic compounds suffered a slight decrease after the use of mentioned materials. Thus, the activated carbon determined the greatest decrease in the concentration of the main phenolic compounds analyzed (gallic acid, protocatechuic acid, gentisic acid, vanillic acid, caffeic acid, *m*-hydroxybenzoic acid), followed by samples treated with carbon nanotubes. When the materials were added after alcoholic fermentation, the content of protocatechuic acid exhibited variations based on the applied treatments: graphene and graphene oxide resulted in an increase in concentration, while treatments with nanotubes and activated carbon led to a decrease in the level of protocatechuic acid.

The concentration of *p*-hydroxybenzoic acid increased following the treatment of wines with carbon-based materials, with the highest amount observed in the sample treated with graphene oxide. Among these materials, it was noted that graphene oxide had an impact on the wine composition by elevating the concentrations of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, vanillic acid, *m*-hydroxybenzoic acid, *p*-coumaric acid, *trans*-resveratrol, and rutin. Wines treated with carbon nanotubes recorded lower values for gallic acid and *m*-hydroxybenzoic acid. In contrast, activated carbon proved to be the most effective in reducing the levels of protocatechuic acid, gentisic acid, catechin, syringic acid, caffeic acid, *m*-hydroxybenzoic acid, *p*-coumaric acid, *trans*-resveratrol, and rutin in wine.

The addition of carbon-based materials in the post-alcoholic fermentation stage significantly influenced the phenolic content of wines.

According to Filipe-Ribeiro et al. [46], activated carbon treatment can be efficient for reducing undesirable odors arising from volatile phenols, such as 4-ethylphenol and 4-ethylguaiacol in wines contaminated with *Dekkera* and *Brettanomyces*. The effectiveness of this treatment relies on the surface area and micropore volume of the activated carbon. It has been observed that higher mesopore surface area and total pore volume

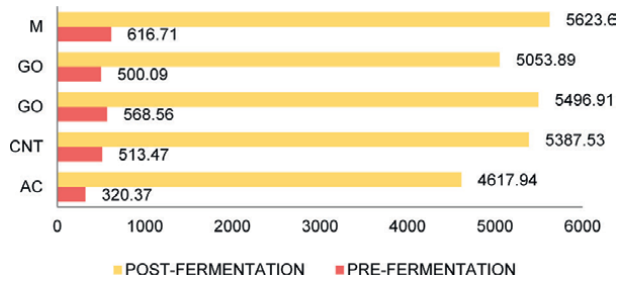

Figure 7.
Total phenolic compounds in wines (Folin-Ciocâlteu method) treated with nanomaterials in pre-fermentation and post-fermentation (b) stages (mg/L gallic acid). M—Untreated sample, G—Graphene, GO—Graphene oxide, CNT—Carbon nanotubes, and AC—Activated carbon.

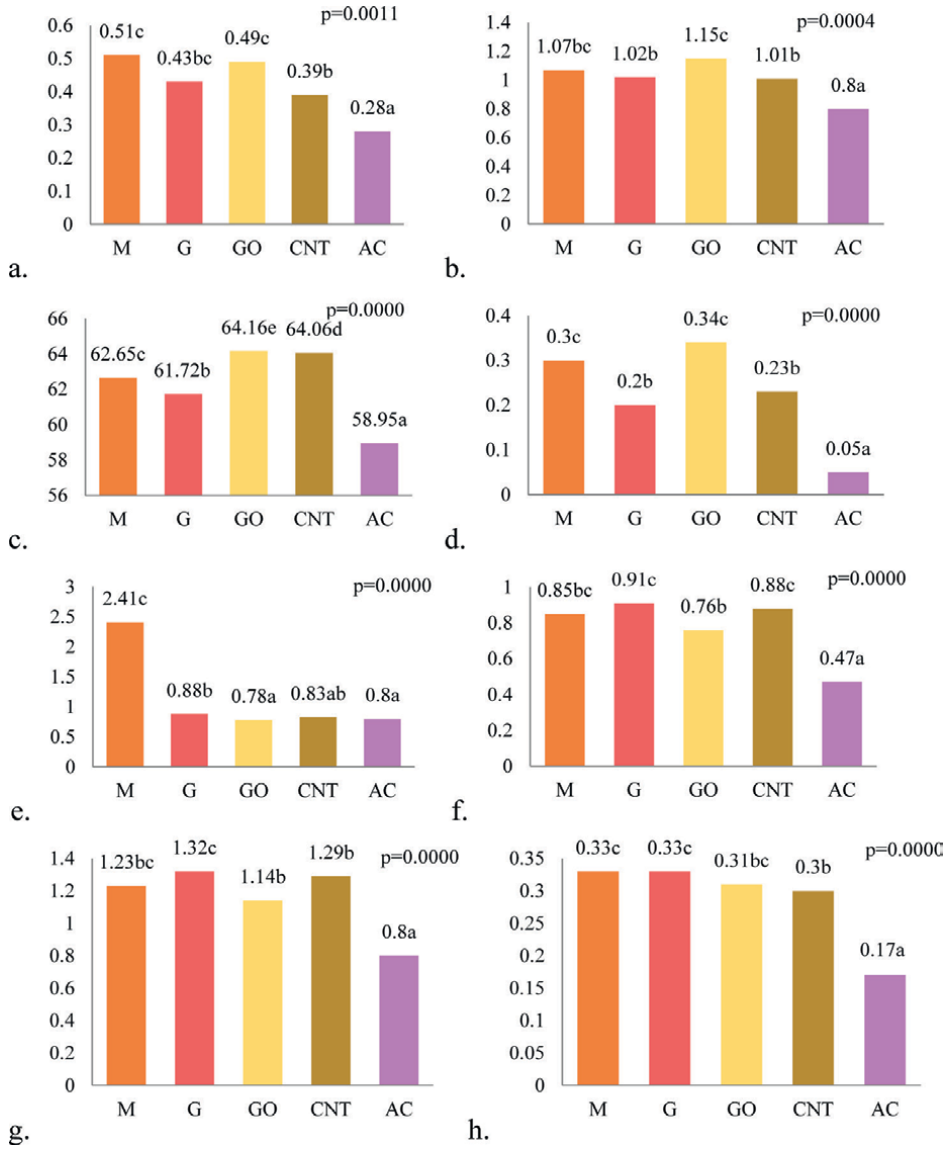

Figure 8.
The impact of nanomaterials on the concentration of phenolic compounds when applied in the pre-fermentation phase (mg/L) gallic acid (a), protocatechuic acid (b), gentisic acid (c), catechin (d), vanilic acid (e), syringic acid (f), caffeic acid (g);,m-hydroxybenzoic acid (h). M—Untreated sample, G—Graphene, GO—Graphene oxide, CNT—Carbon nanotubes, and AC—Activated carbon.

negatively impact anthocyanins and color intensity, while a greater surface area and micropore volume are crucial for the removal of phenolic acids. The successful reduction of volatile phenolics plays a pivotal role in enhancing the positive perception of the fruity attribute in wines. Through a careful selection of the physicochemical characteristics of activated carbon, it becomes feasible to efficiently eliminate volatile phenols without compromising the sensory quality of the wine. This optimal selection ensures that the activated carbon treatment positively contributes to improving the overall quality of the wine without adversely affecting its sensory attributes.

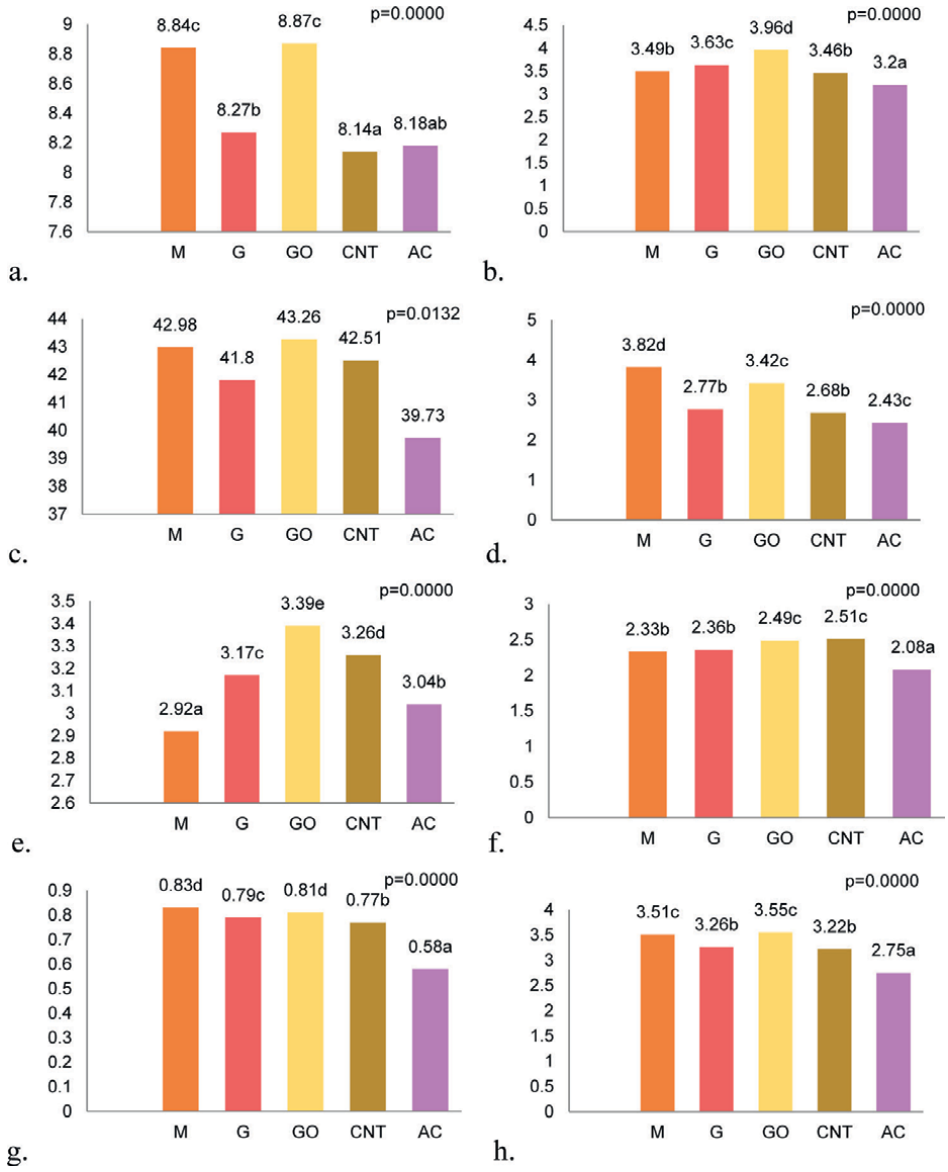

Figure 9.
The impact of nanomaterials on the concentration of phenolic compounds when applied in the post-fermentation phase (mg/L) gallic acid (a), protocatechuic acid (b), gentisic acid (c), catechin (d), vanillic acid (e), syringic acid (f), caffeic acid (g), m-hydroxybenzoic acid (h). M—Untreated sample, G—Graphene, GO—Graphene oxide, CNT—Carbon nanotubes, and AC—Activated carbon.

Cotea et al. [47] chose to evaluate the influence of mesoporous silica material SBA-15 on polyphenols content in Cabernet Sauvignon samples. The authors presented SBA-15 as an efficient alternative for the extraction of phenolic compounds such as quercitin and *cis-* and *trans-*resveratrol from red wine. On this line, similar results were presented for KIT-6 materials in Feteasc ă neagr ă wines [7]. Dumitriu et al. [2] confirm the already mentioned findings and demonstrate that the use of SBA-15 in winemaking leads to significant decrease in the total polyphenol index. In another

study, Luchian et al. [10] obtained a significant rise in concentration of caffeic acids in Cabernet Sauvignon samples treated with KIT-6 (double value compared to the control sample) after 5 months of maturation. Also, the results present a notable increase of rutin content in Merlot samples, when SBA-15 and MCM-41 materials were used. Dumitriu et al. [48] presented a higher retention of phenolic compounds on MCM-41, compared to KIT-6 and SBA-15 in Muscat Ottonel wines. Even if studies on the effect of nanostructured material on phenolic compounds in wine are limited, there are several papers that refer to water and present some essential conclusions. Thus, Pattanaik et al. [49] postulated that higher MCM-41 dosage can lead to better adsorption of phenols while increasing pH values usually generates contrary results. For this study, pH = 5 was the optimum value. In accordance with these results, Kalash et al. [50] confirmed that MCM-41 can be a great alternative in removing 67% of different phenols from contaminated water in different experimental conditions (pH = 4-9, mixing rate = 200 rpm, at room temperature). Also, the authors that the adsorption mechanism fits better with Langmuir isotherm.

The successful application of mesoporous material SBA-15 for the adsorption of phenolic compounds from wine was also proposed by Niculescu et al. [51]. In this study, catechin has the highest retention, followed by rutin, *trans*-cinnamic acid, *trans*-resveratrol, and gallic acid. The authors attributed this adsorption to the dipole moment of these molecules. Moreover, the presence of the electrons in phenyl rings and their availability may increase the interaction with Si-OH groups for the adsorption. This material is proposed as a viable alternative for efficiently extracting *trans*-resveratrol and other significant compounds. According to Anbia & Amirmahmoodi [52], the adsorption isotherm for SBA-15 fits well with the Freundlich equilibrium model.

6. Conclusions

In this chapter, an attempt has been made to cover information regarding phenolic adsorption and its derivatives using various nanocomposites. Minimizing the polyphenolic content in red wines prevents the oxidative browning process while preserving the wine's color. The polyphenolic extract, with its antioxidant properties, could be of interest as pharmaconutrients or as potential food sources, given its positive impact on human health. The utilization of siliceous, aluminosiliceous, and carbon-based materials in wine treatment supports the partial extraction of phenolic compounds and prevents browning and precipitation, thereby enhancing overall stability. Phenolic compounds play a crucial role in wine quality and sensory attributes, and the selective adsorption facilitated by carbon nanotubes could contribute to refining and enhancing the overall characteristics of wines.

Conflict of interest

The authors declare no conflict of interest.

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Chapter 7

Stilbenes: Emerging Applications in Health, Agriculture, and Industry

Sania Raees, Sayed Afzal Shah and Aman Karim

Abstract

Stilbenes are an important class of plant natural products with a core structure made of 1,2-diphenylethylene and exhibit diverse chemical and biological properties. These defensive compounds are produced in grapevines, peanuts, and other plants, and have been a focus of recent research for their therapeutic tendencies. This chapter aims to discuss the distinctive biological activities, emerging applications of stilbenes, and their sustainable use and production. In the pharmaceutical industry, stilbenes are used for their neuroprotective, anticancer, anti-inflammatory, and antioxidant properties. The nutraceutical and food industries are interested in their antiaging effects, metabolic and cardiovascular health impacts. In the agriculture industry, stilbenoids are investigated as biopesticide, as a green alternative to synthetic pesticides. The cosmetic industry uses stilbenes for their UV-protective, skin-brightening, and antiaging potential in skincare and cosmetic products. Besides these, stilbenes are also researched for environment sustainability and industrial applications in food preservation, bioremediation, and polymer science. The chapter also presents sustainable methods of stilbenes production, both biotechnological and synthetic, as well as the challenges associated with stilbenes, such as regulatory difficulties and poor bioavailability, ending with future directions for the applications and usage of stilbenes. The purpose of these discussions is to highlight the growing interest in stilbenes for their natural properties and benefits in health, agriculture, and industry.

Keywords: stilbenes, natural products, pharmaceuticals, cosmeceutical, sustainable production

1. Introduction

Stilbenes are polyphenolic compounds with a core structure made of 1,2,-diphenylethylene, either as a cis or trans isomer, with the latter known for its better stability and biological activity (**Figure 1**). Plants produce stilbenes through the phenylpropanoid pathway and benefit from their role as phytoalexins, that is, natural defense for plants against infections, herbivory, and other environmental stressors such as UV radiations [1, 2]. Resveratrol is the most researched stilbene to date, which is mainly found in berries, peanuts, and grapes. Other known stilbenes identified in various plants include oxyresveratrol, pterostilbene, and piceatannol [3, 4]. To date, many stilbenes have been reported from different plant species and are used in various industries, some of which are shown in **Table 1**. Though naturally available,

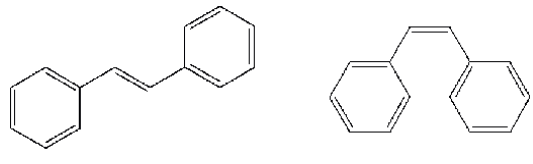


Figure 1.
Basic structure of stilbenes.

researchers have developed sustainable methods to produce these compounds, such as through plant cell culture and microbial fermentation.

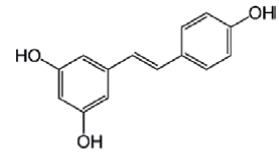
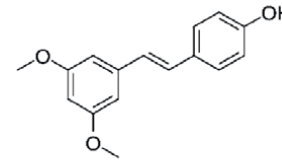
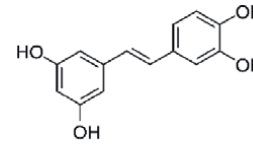
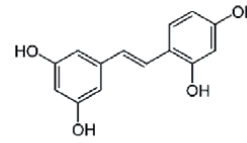
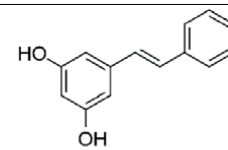
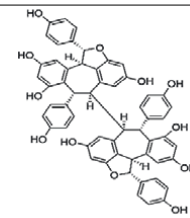
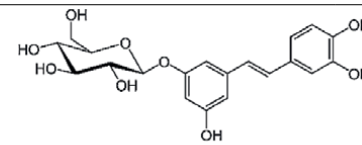
1.1 Historical perspective on stilbene research

Research on stilbenes has been going on for a long time, with a major focus on their occurrence in plants and chemical structures, but the significance of their biological activity became the center of attention after the discovery of their role as phytoalexins in the mid-twentieth century [6, 27, 28]. In the 1900s, interest spiked in the medicinal potential of stilbenes after a remarkable discovery during the French Paradox, where resveratrol found in red wine was associated with a reduction in cardiovascular risks [29, 30]. After that, further aspects were explored, such as bioavailability enhancements, synthetic derivatives, and applications in various industries. On top of that, the development of sustainable methods for their extraction and metabolic engineering has added to the depth of research on stilbenes [31, 32].

1.2 Importance of stilbenes in natural product research and applications

Stilbenes have surfaced as key bioactive compounds with potential applications in diverse industries, from pharmaceuticals to nutraceuticals, agriculture, cosmetics, environmental science, and industrial sectors (**Figure 2**).

Their broad biological properties and advancements in extraction and synthetic methods have led to the potential value of stilbenes as natural compounds beneficial for a wide range of purposes. Stilbenes have shown significant antioxidant, anti-inflammatory, and anticancer activities in the pharmaceutical sector [31, 33–35]. The most well-studied stilbene, resveratrol, has been proven to have neuroprotective activities against neurodegenerative diseases, such as Alzheimer's and Parkinson's. Pterostilbene, on the other hand, is a structurally modified analog of resveratrol that has been shown to have increased bioavailability, thus becoming a potential therapeutic agent for a variety of diseases [36–38]. The natural antioxidant properties of stilbenes also make them crucial in nutraceuticals and functional foods. They have been employed extensively in food fortification for cardiovascular health, metabolic regulation, and antiaging benefits [39, 40]. However, because of low stability and solubility, innovative techniques like nano-encapsulation are applied to improve bioavailability and efficacy in the dietary supplement area [41, 42]. As natural pesticides and plant defense molecules, stilbenes in agriculture diminish the requirement for synthetic agrochemicals. Plant resistance to fungal pathogens and environmental stressors is enhanced by these compounds, thus providing an eco-friendly alternative for sustainable farming [43, 44]. Advances in genetic engineering have also enabled the improvement of stilbene biosynthesis in crops, further strengthening their role

No.	Stilbenes	Plant source	Chemical structure	Refs.
1.	Resveratrol	*Paeonia suffruticosa* Andr. Var. papaveracea (Andr.) Kerner *Ananas comosus* (L.) Merr. *Magnolia officinalis* Rehder & E. Wilson (Houpo) *Olea europaea* Linnaeus *Pistacia vera* Linnaeus	 $C_{14}H_{12}O_3$	[5–9]
2.	Pterostilbene	*Vaccinium corymbosum* Linnaeus *Pterocarpus marsupium* Roxburgh *Vaccinium angustifolium* Aiton	 $C_{16}H_{16}O_3$	[10–12]
3.	Piceatannol	*Caragana tibetica* (Maxim. ex C.K. Schneid.) Kom. *Vitis vinifera* Linnaeus	 $C_{14}H_{12}O_4$	[13–16]
4.	Oxyresveratrol	*Artocarpus lacucha* Buch.-Ham.	 $C_{14}H_{12}O_4$	[17]
5.	Pinosylvin	*Pinus densiflora* Jane Kluis *Pinus sylvestris* Linnaeus	 $C_{14}H_{12}O_2$	[18, 19]
6.	Hopeaphenol	*Shorea uliginosa* Foxw. *Vitis vinifera* Linnaeus *Dryobalanops lanceolata* Burck.	 $C_{56}H_{42}O_{12}$	[20–22]
7.	Astringin	*Picea sitchensis* (Bong.) Carr.	 $C_{20}H_{22}O_9$	[23]

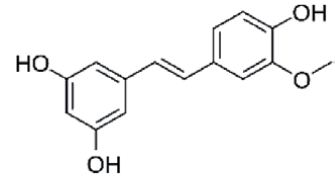
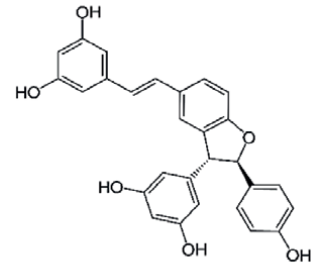
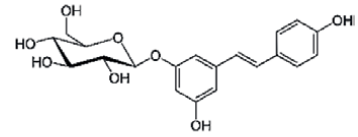
No.	Stilbenes	Plant source	Chemical structure	Refs.
8.	Isorhapontigenin	*Gnetum pendulum* C.Y. Cheng *Caragana tibetica* (Maxim. ex C.K. Schneid.) Kom.	 $C_{15}H_{14}O_4$	[16, 24]
9.	Viniferin (ε-Viniferin)	*Vitis vinifera* Linnaeus	 $C_{28}H_{22}O_6$	[13–16]
10.	Polydatin	Paeonia ostii T. Hong and J. X. Zhang	 $C_{20}H_{22}O_8$	[25, 26]

Table 1.
A list of stilbenes, their source plants, and chemical structures.

in plant protection and productivity [43, 45, 46]. Cosmetics industries utilize stilbenes for their antiaging, anti-inflammatory, and skin-brightening properties. Their potential to inhibit tyrosinase activity makes them useful in hyperpigmentation, while their photoprotective properties help avoid UV-induced damage to the skin [31, 35]. In response to growing consumer interest in natural skincare products, stilbenes are being increasingly used in cosmetic formulations [31, 47]. Other than health-related uses, stilbenes have also been used in the environmental and industrial fields. They have been explored for their roles in bioremediation, wastewater treatment, natural dyes, and fluorescent agents within materials science. In addition, their ability to prevent oxidation makes them suitable for the elongation of the shelf life of food products, as well as the stabilization of sensitive materials [28, 48, 49]. In addition, the sustainable production and utilization of stilbenes are growing in popularity, with biotechnology developments into large-scale, environmentally friendly production processes. Plant cell culture, microbial fermentation, and waste from agriculture valorization methods give opportunities for a more sustainable production alternative compared to traditional extraction and potentially stabilize the supply of such important compounds [31, 50–52]. **Table 2** shows a list of some of the naturally occurring stilbenes known so far and their sources.

This chapter explores diverse and emerging applications of stilbenes, highlighting their potential as natural bioactive compounds with significant benefits for human

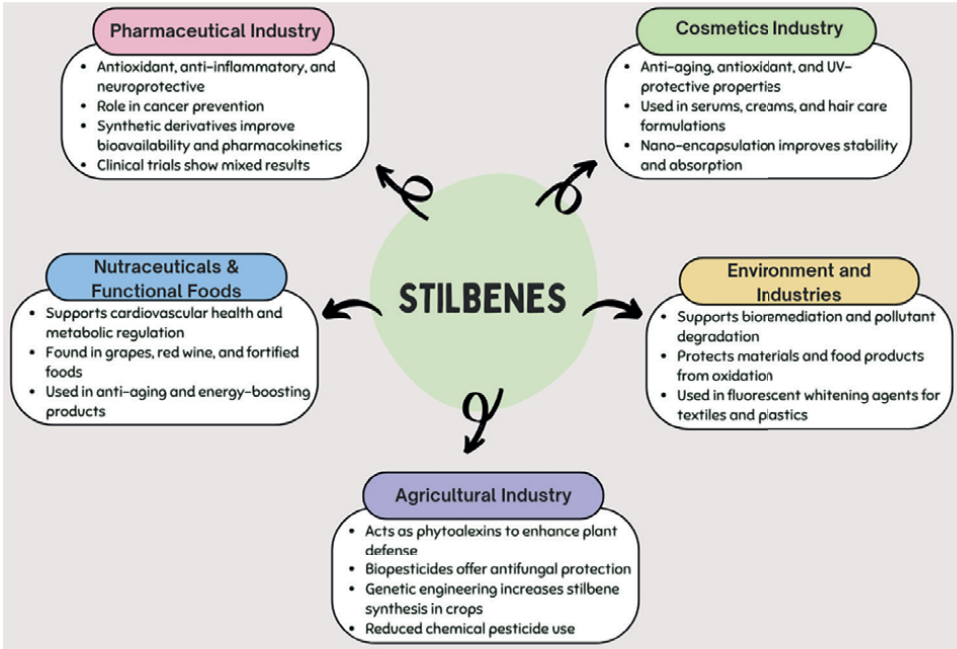


Figure 2.
A summary of diverse roles of stilbenes in various industries.

health, sustainable agriculture, and industrial innovation. As research progresses, the continued exploration of stilbenes in various fields will contribute to discoveries and improved applications, further expanding their impact on science and industry.

2. Biological and medicinal properties of stilbenes

Stilbenes are a unique class of polyphenols that exhibit a plethora of biological and medicinal properties. These compounds are extensively studied for their antioxidant, anti-inflammatory, antimicrobial, cardioprotective, neuroprotective, and anticancer activities (**Figure 3**). Their potential therapeutic applications have made them promising candidates in drug development and disease prevention strategies [1, 35, 57].

2.1 Antioxidant and anti-inflammatory properties

Potent antioxidant activity is one of the most extensively studied properties of stilbenes. Stilbenes, resveratrol in particular, scavenge reactive oxygen species (ROS) and prevent oxidative stress-inducing cellular damage, which is a major factor in aging and chronic diseases. The ability of stilbenes to activate endogenous antioxidant pathways, such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, has been highlighted as a critical mechanism for their protective effects against inflammation and oxidative damage [1, 39, 58]. Stilbenes also exhibit anti-inflammatory effects by inhibiting pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These anti-inflammatory properties make stilbenes valuable in managing conditions such as arthritis, cardiovascular disease, and neuroinflammation [1, 59].

No.	Natural stilbenes	Sources	References
1.	Resveratrol	Grapes Red wine Peanuts Berries *Polygonum cuspidatum*	[8]
2.	Pterostilbene	Blueberries Grapes *Pterocarpus marsupim*	[53]
3.	Piceatannol	Grapes Passion fruit White tea Rhubarb	[54]
4.	Pinosylvin	Pine heartwood Scots pine (Pinus sylvestris)	[55]
5.	Viniferins	Grapevines Red wine	[56]

Table 2.
Various sources of naturally occurring stilbenes.

2.2 Cardioprotective and neuroprotective effects

Stilbenes contribute to cardiovascular health by modulating lipid metabolism, improving endothelial function, and reducing hypertension. Studies show that resveratrol can prevent atherosclerosis by inhibiting LDL oxidation and enhancing nitric oxide (NO) production, which promotes vasodilation and improves circulation [60, 61]. In neurodegenerative diseases, stilbenes offer protection against neuronal damage by reducing oxidative stress, inhibiting amyloid-beta aggregation, and modulating neurotransmitter pathways. Their neuroprotective effects are being explored in conditions such as Alzheimer's and Parkinson's disease, where they have been shown to enhance cognitive function and delay disease progression [62–64].

2.3 Anticancer properties

Stilbenes have attracted significant attention for their anticancer properties. These compounds regulate multiple signaling pathways involved in cancer progression, including apoptosis induction, cell cycle arrest, and inhibition of metastasis. Resveratrol and its derivatives have been studied for their ability to sensitize cancer cells to chemotherapy and radiotherapy, making them valuable adjuncts in cancer treatment [65]. Furthermore, the structural modifications of stilbene derivatives have been explored to enhance their bioavailability and potency as anticancer agents. Medicinal chemistry approaches have led to the development of synthetic stilbene analogs with improved pharmacokinetic profiles, expanding their therapeutic potential [24].

2.4 Antimicrobial and antiviral effects

Stilbenes also demonstrate antimicrobial properties, inhibiting the growth of bacteria, fungi, and viruses. Studies suggest that resveratrol and pterostilbene exhibit strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* while

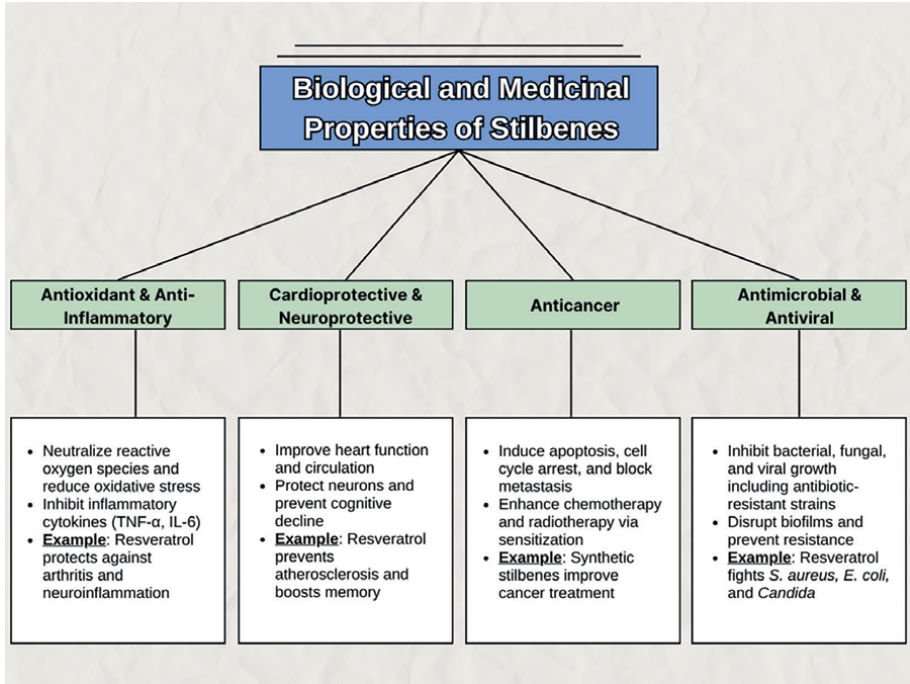


Figure 3.
Summary of biological and medicinal properties of stilbenes.

also showing antifungal effects against *Candida albicans* [66–69]. The ability of stilbenes to interfere with microbial biofilms further enhances their role in combating antibiotic-resistant infections [65]. Additionally, stilbenes have shown promise as antiviral agents, particularly against influenza and coronaviruses. Their immunomodulatory effects help in reducing viral replication and inflammatory responses, suggesting their potential use in antiviral therapies [70, 71].

3. Stilbenes in pharmaceuticals

3.1 Therapeutic potential of stilbenes

Stilbenes, especially resveratrol and its derivatives, have gained significant attention for their multi-target therapeutic potential across various diseases. As natural polyphenols, they possess potent antioxidant and anti-inflammatory properties, making them highly effective in mitigating oxidative stress and inflammation-related disorders. For example, in neurodegenerative diseases such as Alzheimer's and Parkinson's, resveratrol has shown remarkable neuroprotective effects. It improves oxidative damage, promotes neurogenesis, and enhances mitochondrial function, which is very important for maintaining neuronal health [36, 38, 72]. These findings highlight its potential as a preventive and therapeutic agent for slowing disease progression in conditions marked by neurodegeneration. In oncology, the anticancer properties of stilbenes are particularly noteworthy. Natural stilbenes, including resveratrol, pterostilbene, and piceatannol, have demonstrated their ability to regulate multiple molecular pathways, such as modulating DNA methylation and

gene expression [73–75]. This ability to reverse aberrant epigenetic changes positions them as promising agents for cancer prevention and treatment. Specifically, colorectal cancer therapy has benefited from the discovery that stilbenes can influence DNA methylation machinery, reducing tumor growth and progression with minimal side effects [76, 77]. Another emerging area of research involves the therapeutic potential of cannabis-derived stilbenes. These compounds exhibit a wide spectrum of biological activities, including anti-inflammatory, antiviral, and antioxidant effects. A recent study found stilbenes in cannabis, many of which have shown promise in predictive pharmacological modeling for therapeutic applications [78, 79].

3.2 Stilbene-derived drugs and clinical trials

The pharmaceutical development of stilbene-based drugs has advanced significantly, focusing on enhancing their bioavailability and therapeutic efficacy. Resveratrol remains the most studied compound, but due to its low bioavailability, researchers are more interested in synthetic derivatives like pterostilbene and modified analogs. These derivatives have been shown to possess improved pharmacokinetic profiles, including better absorption and metabolic stability [4, 80]. This progress highlights the potential of synthetic biology and medicinal chemistry in addressing the limitations of natural stilbenes. Clinical trials researching the efficacy of resveratrol found mixed results. Some studies support its neuroprotective effects in degenerative diseases, while others report inconsistent outcomes due to variable dosage, delivery methods, and patient population [3, 81–83].

4. Stilbenes in nutraceuticals and functional foods

4.1 Health benefits of stilbene-enriched foods

Stilbenes, especially resveratrol and pterostilbene, are prominent in nutraceuticals due to their remarkable health-promoting properties. These polyphenols are known to support cardiovascular health by improving endothelial function, reducing inflammation, and modulating lipid profiles. Resveratrol, found in red wine and grapes, is one of the most studied stilbenes in this regard. It has been shown to improve arterial flexibility and mitigate oxidative damage, which are critical for preventing atherosclerosis and other cardiovascular diseases [1, 39, 77]. Additionally, stilbenes exhibit antiaging effects through their ability to activate sirtuins, a class of proteins that regulate cellular health and longevity. These effects are increasingly being leveraged in functional foods targeting age-related metabolic disorders such as diabetes and obesity. Incorporating stilbenes into daily diets, either through naturally rich foods or fortified products, offers a preventive approach to managing these conditions [39, 84, 85].

4.2 Food fortification with stilbenes

Stilbenes are incorporated into functional foods to enhance their health benefits. Techniques such as nano-encapsulation and polymeric coating have been employed to improve the stability and bioavailability of stilbenes in food matrixes [86]. For instance, recent advancements in nanoparticle-based delivery systems have shown significant promise in maintaining the stability of stilbene-glycosides, even under challenging

Sr.	Functional food	Stilbene used	Purpose of enrichment	References
1.	Red wine	Resveratrol	Antioxidant Cardiovascular health Antiaging	[89, 90]
2.	Grape juice	Resveratrol	Improve endothelial function Reduces oxidative stress	[90]
3.	Peanut butter	Resveratrol	Enhance shelf life Provide antioxidant benefits	[91]
4.	Dark chocolate	Resveratrol	Improves cardiovascular health Enhance antioxidant capacity	[92]
5.	Blueberry supplements	Pterostilbene	Cognitive health Anti-inflammatory Blood sugar regulation	[12]
6.	Fortified cereals	Resveratrol	Promote heart health Provide antioxidant support	[93]
7.	Green tea extracts	Resveratrol	Synergistic antioxidant effects Enhance metabolic health	[28, 94, 95]
8.	Energy bars	Resveratrol	Boost energy Provide antioxidant and anti-inflammatory benefits	[96, 97]
9.	Dietary supplements	Resveratrol Pterostilbene	Antiaging Improves mitochondrial function Supports cardiovascular health	[28, 98]
10.	Fortified yogurt	Resveratrol	Improves gut health Provide antioxidant benefits	[99, 100]

Table 3.
Commercially available functional foods with stilbene enrichment.

environmental conditions like heat and UV exposure. These technologies ensure sustained release and greater absorption in the human body, increasing the efficacy of the fortified food products [87, 88]. Fortified foods such as energy drinks, beverages, and dairy products enriched with resveratrol and pterostilbene have gained popularity among health-conscious consumers; examples of such foods are shown in **Table 3**.

5. Agricultural applications of stilbenes

5.1 Plant defense mechanisms

Stilbenes play a pivotal role in plant defense, serving as phytoalexins synthesized in response to various environmental stressors, such as pathogen attacks, drought, salinity, and extreme temperatures. Resveratrol and pterostilbene are among the most studied stilbenes, demonstrating significant antimicrobial and antioxidant properties. These compounds not only act directly by inhibiting microbial growth but also indirectly by triggering defense signaling pathways in plants. For example, stilbenes activate pathways associated with phytohormones such as auxins, gibberellins, and abscisic acid, which are critical for plant stress adaptation and survival. A study on *Arabidopsis thaliana* found that the exogenous application of resveratrol significantly improved resistance to heat stress and soil salinity, highlighting the potential of stilbenes to improve plant resilience

[101]. Stilbenes also contribute to the structural reinforcement of plant cell walls, making them less susceptible to pathogen invasion. Their phenolic nature allows them to cross-link with other cell wall components, creating a physical barrier against microbial penetration. In addition, these compounds can act as antioxidants, neutralizing reactive oxygen species (ROS) generated during stress conditions [43, 101].

5.2 Stilbene-based biopesticides

The use of stilbenes as natural biopesticides has gained traction due to their dual action against pests and pathogens. Stilbene-enriched extracts from grapevines and other plants have demonstrated potent antifungal activity against pathogens like *Botrytis cinerea* and *Plasmopara viticola*, both of which are significant threats to agricultural crops. These extracts work by disrupting the cellular membranes of fungi, inhibiting their growth and reproduction [102, 103]. A study on grapevine roots identified stilbene oligomers, such as vitisin A and B, as the most effective compounds for pest control. These oligomers not only reduced pest infestations but also prompted crop health by boosting the plant's innate immune responses [44]. Stilbene-based pesticides have several advantages over synthetic chemicals. They are biodegradable, pose minimal risk to nontarget organisms, and leave no harmful residues in the environment. For example, a grapevine-derived stilbene extract was shown to effectively protect plants from fungal infections without affecting beneficial soil microorganisms. The formulation of such extracts into sprays or powders offers practical solutions for farmers seeking sustainable pest management options [44, 104].

5.3 Genetic engineering for enhanced stilbene production

Advances in genetic engineering have paved the way for the development of crops with enhanced stilbene production, providing an effective strategy for improving plant resilience and reducing reliance on chemical pesticides. Genetic modifications target key enzymes in the phenylpropanoid pathway, such as stilbene synthase (STS), to upgrade stilbene biosynthesis. Transgenic grapevines expressing multiple STS genes have been shown to accumulate higher levels of resveratrol and related compounds, significantly increasing their resistance to fungal pathogens [43, 45]. Biotechnology is also being used to produce stilbenes in non-native hosts such as *Saccharomyces cerevisiae* and *Escherichia coli*. The microbes are engineered to express the entire stilbene pathway, allowing for large-scale production of stilbenes under controlled conditions. This approach ensures a consistent supply of these valuable compounds and reduces dependency on plant-derived sources, which can be influenced by environmental factors. CRISPR-Cas9 technology has further revolutionized the field by enabling precise editing of genes involved in stilbene biosynthesis [105, 106]. This technique allows researchers to improve specific traits like pest resistance or drought tolerance in crops.

6. Stilbenes in cosmetics

6.1 Skin care benefits of stilbenes

Stilbenes such as resveratrol, piceatannol, and oxyresveratrol have become vital ingredients in the cosmetics industry due to their potent antioxidant,

anti-inflammatory, and antiaging properties. These compounds neutralize free radicals and inhibit oxidative stress, which are the main reasons for skin aging and UV-induced damage. Resveratrol has shown significant efficacy in numerous studies against wrinkles and skin elasticity and in promoting collagen synthesis [31, 107]. Also, stilbenes exhibit anti-tyrosinase activity, which is crucial for reducing hyperpigmentation and achieving an even skin tone. Oxyresveratrol has shown promising results as a natural skin-lightening agent, which made it a popular ingredient in formulations for melasma and other pigmentation disorders. The photoprotective properties of stilbenes signify their application further in sunscreen formulations by preventing UV-induced damage and inflammation [108, 109]. A list of some stilbenes used in cosmetic and skincare products is given in **Table 4**, along with the reason they are employed.

6.2 Formulation and delivery

The integration of stilbenes into cosmetic formulations has been a challenge because of their poor water solubility and stability when exposed to light or air. Recent developments in nanotechnology have addressed these issues by employing nano-encapsulation and microemulsion systems. For example, encapsulating resveratrol in liposomes or polymeric nanoparticles improves its stability and allows its controlled release, ensuring prolonged activity on the skin [117, 118]. Cosmetic products like antiaging creams, serums, and sunscreens are increasingly using these advanced delivery systems to maximize the bioavailability of stilbenes. Furthermore, green technologies like natural deep eutectic solvents (NADES) are being explored for sustainable extraction and formulation of stilbenes, which aligns with the growing demand for eco-friendly cosmetic products [52, 119, 120].

6.3 Emerging trends in stilbene cosmetics

The cosmetics market has experienced an increase in demand for products that use natural and plant-based ingredients with stilbenes at the core, owing to their

Sr.	Stilbenes	Uses	References
1.	Resveratrol	Antioxidant Antiaging Brightening Reduces hyperpigmentation Protects against UV damage	[110, 111]
2.	Pterostilbene	Anti-inflammatory Antiaging Skin-soothing	[11, 112]
3.	Oxyresveratrol	Skin-brightening Tyrosinase inhibition Reduction of dark spots	[113]
4.	Piceatannol	Antioxidant Antiaging Protection against environmental stressors	[114]
5.	Hopeaphenol	Antiaging Skin firming Reduction of fine lines and wrinkles	[115, 116]

Table 4.
Stilbenes in cosmetics/skincare and their applications.

multifunctional properties. Resveratrol-based products are particularly popular among consumers seeking natural alternatives to synthetic antioxidants and antiaging agents. Brands are also focused on using stilbenes in combination with other bioactive ingredients like hyaluronic acid and peptides to make multifunctional skincare products [31, 121–123]. Also, stilbenes are being used to make hair care products because of their antioxidant properties, which help protect and strengthen hair against environmental damage. Furthermore, the cosmeceuticals industry, that is, cosmetics with medical uses, has been using stilbenes to address skin conditions like acne, eczema, and rosacea [31, 109]. Consumers' preferences for sustainability have also driven innovations in sourcing and producing stilbenes. Techniques like plant cell culture and microbial fermentation are being used in a more sustainable way to reduce dependence on seasonal plant sources [52, 124].

7. Environmental and industrial applications

7.1 Environmental benefits

Stilbenes have shown potential in environmental applications like in bioremediation and as protective agents in stabilizing sensitive materials. Natural stilbenes like resveratrol and piceatannol neutralize reactive oxygen species (ROS), which play a critical role in environmental oxidation processes. Recent studies show that stilbenes can protect light-sensitive foods from oxidation by quenching singlet oxygen, thus increasing the shelf life of these products [28, 59, 101, 109, 125]. Also, stilbene-based compounds can be useful in water treatment processes. Their antioxidant properties help with the degradation of pollutants, while their nontoxic nature helps reduce secondary contamination. Advanced oxidation techniques that use stilbenes or their derivatives show promise in treating industrial effluents, reducing toxicity, and improving water quality [50, 80, 126].

7.2 Industrial applications

Stilbenes have numerous industrial applications owing to their photophysical and chemical properties. They are widely used in manufacturing fluorescent whitening agents, which improve the appearance of textiles, paper, and plastics by improving their brightness and whiteness. The unique fluorescence and photoisomerization capabilities of stilbenes make them suitable for optical and electronic industrial products like organic lasers and nonlinear optical devices [28, 35, 50]. Another emerging use of stilbenes is in catalysis, where materials made of stilbenes allow environmentally friendly reactions. For example, silica-supported stilbene catalysts have been used for efficient E-stilbene synthesis under mild conditions, addressing the need for sustainable industrial practices [127, 128].

8. Sustainable production and utilization of stilbenes

8.1 Natural sources and extraction

Stilbenes like resveratrol and its derivatives are mainly extracted from plants like grapevines (*Vitis vinifera*) and mulberry (*Morus alba*). These compounds are

highly valued for their bioactive properties, which makes them very important in the pharmaceutical, nutraceutical, and cosmetic industries [129, 130]. However, traditional cultivation methods often result in inconsistent yields due to environmental and seasonal factors. In response, sustainable strategies such as *in-vitro* root culture systems have been developed. For example, root cultures of *Morus alba* optimized with precursor feeding and elicitors like methyl jasmonate and yeast extract have shown enhanced stilbene production, achieving high yields of resveratrol and oxyresveratrol [131]. Subcritical water extraction (SWE) has emerged as a green chemistry method for isolating stilbenes from grapevine by-products. SWE uses high-pressure water as a solvent to extract compounds efficiently without organic solvents, making it eco-friendly and scalable for industrial use [132].

8.2 Synthesis and biotechnological production

Biotechnological advances have revolutionized the production of stilbenes, providing alternatives to plant-based extraction. Microbial systems such as engineered yeast and bacteria are now used for stilbene biosynthesis [133]. These systems use renewable feedstocks like glucose and glycerol for fermentation, enabling consistent and scalable production of resveratrol and its derivatives. For example, the cytochrome p450 enzyme system in *Thermobifida fusca* has been engineered to hydroxylate stilbenes efficiently, yielding high titers of bioactive compounds [134, 135]. Plant cell culture systems like hairy root cultures have also been developed for the sustainable production of stilbenes. Grapevine and peanut hair roots have been shown to produce high levels of resveratrol and prenylated stilbenes upon elicitation with methyl jasmonate and other agents [136, 137].

8.3 Circular economy and waste valorization

The use of agricultural and industrial by-products for stilbene extraction is gaining attention as a sustainable practice aligned with the principles of a circular economy. Grapevine waste (canes, roots, and leaves) is a rich source of stilbenes that can be repurposed for high-value applications. Recent studies have demonstrated that using natural deep eutectic solvents (NADES) for ultrasonic-assisted extraction yields high levels of stilbenes while maintaining their stability over extended periods [138–140]. Furthermore, biotechnological innovations allow for the valorization of these by-products into nutraceuticals and cosmeceuticals. Extracts from grapevine co-products enriched in stilbenes have been shown to exhibit antifungal properties, offering sustainable alternatives to synthetic pesticides [141, 142].

9. Challenges in application of stilbenes

One of the primary limitations of natural stilbenes, such as resveratrol, is their poor bioavailability. Stilbenes are rapidly metabolized in the liver and intestines, leading to low systemic concentrations after oral administration. Additionally, their hydrophobic nature limits their solubility in aqueous environments, reducing their absorption in the gastrointestinal tract. These factors collectively hinder their therapeutic efficacy in clinical applications [65]. To address these issues, researchers are developing various delivery systems, including nano-encapsulation, liposomal

formulations, and cyclodextrin complexes, to enhance the stability and bioavailability of stilbenes. Encapsulation techniques protect these compounds from rapid degradation, allowing for sustained release and improved absorption [1].

9.1 Synthetic and biotechnological challenges

While natural sources of stilbenes provide a sustainable supply, their production is limited by environmental factors, seasonal variations, and low yield from plant tissues. Alternative strategies, such as microbial fermentation and plant cell cultures, have been explored to produce stilbenes on a larger scale. However, optimizing these biotechnological processes for commercial production remains a challenge due to the need for high-yield metabolic engineering and cost-effective bioreactor systems [133]. Synthetic chemistry has also been employed to modify stilbene structures, enhancing their pharmacokinetic properties and biological activity. However, the synthesis of highly potent derivatives while maintaining safety and low toxicity requires extensive structure–activity relationship (SAR) studies. Future research should focus on developing more efficient synthetic routes for producing novel stilbene analogs with improved medicinal properties [65].

9.2 Regulatory and market challenges

Despite their promising bioactivity, the regulatory approval of stilbene-based pharmaceuticals and nutraceuticals faces hurdles due to inconsistencies in clinical data and standardization issues. The variability in extraction methods, purity levels, and formulation stability complicates the establishment of regulatory guidelines. Additionally, clinical trials on stilbenes have yielded mixed results, with some studies reporting inconsistent therapeutic effects due to differences in dosage, patient populations, and study design. Consumer awareness and market acceptance of stilbene-based products also play a critical role in their commercialization. While the demand for natural bioactive compounds is rising, the cost of high-purity stilbene formulations may limit their accessibility. Strategic collaborations between academia, industry, and regulatory bodies are necessary to accelerate the translation of stilbene research into viable commercial products [1].

10. Future directions in stilbene research

Looking ahead, future research on stilbenes should focus on several key areas to maximize their therapeutic and industrial potential. The development of novel derivatives through medicinal chemistry approaches is crucial for enhancing the stability, potency, and specificity of stilbene compounds for targeted disease treatment. Structural modifications and synthetic analogs could lead to more effective and bioavailable stilbene-based drugs. Additionally, advancements in drug delivery systems, such as nano-formulations, transdermal patches, and injectable suspensions, can significantly improve the absorption and therapeutic efficiency of stilbenes, addressing their current limitations related to bioavailability and metabolism. The integration of stilbenes into personalized medicine represents another promising avenue, where these compounds could be tailored to target specific metabolic pathways and genetic profiles. Precision medicine applications could enhance the effectiveness of stilbene-based therapies in treating conditions such as neurodegenerative

diseases, cancer, and cardiovascular disorders. Sustainable production methods also need further optimization, with microbial fermentation, metabolic engineering, and plant tissue culture techniques offering scalable and environmentally friendly alternatives to traditional extraction methods. These approaches would ensure a consistent supply of high-quality stilbenes while reducing reliance on seasonal plant sources. Furthermore, large-scale, well-controlled clinical trials are essential to validate the efficacy of stilbenes across various therapeutic applications and facilitate regulatory approvals. Standardized studies assessing dosage, long-term effects, and potential drug interactions will be critical for the successful transition of stilbenes from experimental research to widely accepted medical and industrial applications. With continued advancements in these areas, stilbenes could emerge as vital components of next-generation pharmaceuticals, functional foods, and sustainable industrial materials, unlocking their full potential for human health and environmental sustainability.

11. Conclusion

Stilbenes have emerged as highly valuable natural compounds with extensive applications across multiple industries. Their potent biological activities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial properties, have positioned them as promising candidates in pharmaceutical and nutraceutical formulations. In agriculture, their role as natural pesticides and plant defense molecules contributes to more sustainable crop protection strategies. The cosmetics industry has also embraced stilbenes for their antiaging and photoprotective effects, while industrial sectors are exploring their potential in bioremediation and sustainable material production. Despite their immense potential, challenges such as limited bioavailability, stability issues, and regulatory barriers continue to hinder the widespread adoption of stilbene-based products. However, advancements in biotechnology, nano-formulation, and synthetic chemistry are paving the way for more effective and scalable production methods. Future research should focus on optimizing these strategies, conducting rigorous clinical trials, and integrating stilbenes into personalized medicine and sustainable industrial applications. Overall, the growing interest in stilbenes underscores their significance as multifunctional bioactive compounds. Continued interdisciplinary collaboration between scientists, industry leaders, and policymakers will be essential in harnessing the full potential of stilbenes for human health, environmental sustainability, and industrial innovation.

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Conflict of interest

The authors declare no conflict of interest.

Declaration of use of AI

An AI tool, ChatGPT, was used only to rewrite the manuscript for better clarity of the sentences. AI was not used to generate any data for this manuscript. The authors have reviewed the entire manuscript after using AI tools to validate the authenticity of the contents.

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Phenolic compounds have received considerable attention from the scientific community due to their presence in plants and other natural sources, as well as their antioxidant, antimicrobial, and anti-inflammatory properties, which have led to increasing interest in functional foods, the pharmaceutical sector, agriculture, and sustainable materials. This book presents the reader with several chapters on the most recent achievements in the extraction, identification, and application of phenolic compounds from natural sources. It explores both conventional and emerging technologies, such as green extraction methods and nanotechnology, while addressing challenges related to bioavailability, stability, and industrial integration. The book presents an integrated approach to transforming agro-industrial by-products into high-value, phenolic-rich ingredients, with a specific focus on modern extraction technologies, eco-friendly preparative processes, and a promising perspective for applications. Provided by leading experts from a broad spectrum of disciplines, including natural product chemistry, food science, biotechnology, and applied sustainability, this book is an excellent tool for researchers, students, and professionals seeking to understand the diverse and effective bioactive compounds and their emerging applications in various industries.

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