



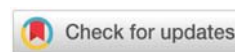
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Review Article

Recent studies on Polyphenols: Extraction technologies and prospects

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Abstract

Polyphenols constitute a group of the most important bioactive compounds widely represented naturally in many plant species. Fresh plant tissues, plant residues, and agro-wastes contain consequent amounts of polyphenolic compounds. In recent years, there is a developing interest in utilizing these bioactive compounds for nutraceutical and pharmaceutical applications due to their beneficial effects on human health and their potential in improving the quality of food. However, their effectiveness is depending on many factors such as the extraction technique, the plant tissue, and species origin, environmental factors, the solvent used for the extraction, etc. The purpose of this critical review is to address and give an insight into the latest review and research articles regarding extraction methods and highlights the prospects of bioactive compounds found in several plant tissues.

Introduction

Nowadays, it is agreed that there is a growing demand for antioxidant compounds that can be used as dietary supplements in human and animal nutrition [1-3], as functional food [4], to improve beverages and wellness [5] and as personal care products in pharmaceuticals and cosmeceuticals [6]. These phytochemicals are also segmented on basis of the form used (powder or liquid derivatives) and their functionality (carotenoids, phytosterols, flavonoids, etc.). Previous studies on phytochemicals recovery have focused primarily on waste from individual fruits and vegetables [7,8], the farm produces [9,10] and waste from processing industries [11,12].

Natural phenol polymers are contained in several, different origin sources such as plant tissues, consumed foods, non-dietary sources, agro-wastes, and food by-products. Among them, agro-wastes that are a source of valuable compounds, such as polyphenols, have stimulated the last few years of intense research work aimed at documenting the many

favorable properties of these materials and developing applications due to protection and benefits of public health and the environment.

In particular, Patra, et al. [13], briefly discuss the different technologies, mechanisms, processes, advantages, disadvantages, characteristics of the extracted bioactive compounds, and process parameters used to extract bioactive compounds from fruit industry by-products such as seeds and peels.

Furthermore, according to Kurek, et al. [14], although technologies based on new principles without toxic solvents have been developed, many technical signs of progress are still needed. In particular, Dorosh, et al. [15], in their review, comprise studies of the last two decades by discussing the advantages and disadvantages of conventional and environmentally friendly technologies used for the extraction of vine-cane phenolic compounds. Depending on the technique used and the extraction conditions, the amount and the profile

of phenolic compounds are affected. More specifically, the authors concluded that due to the different variables such as temperature, time, applied pressure, extraction solvent, solid-to-liquid ratio, and plant variety it is to make a direct comparison between the applied methods and select the best one. Thus, they concluded that a standard method cannot be used for the characterization of the extracts.

The purpose of this review article is to mention some of the most recent research studies on extraction technologies and prospects regarding bioactive substances of polyphenolic compounds.

Extraction technologies

Many different parameters such as temperature, extraction time, amount of solvent used, type of solvent, and part of the

plant tissue used -whole fruit, pulp, or waste- are involved in the polyphenol extraction process in order to assess total polyphenol content (TPC) and antioxidant activity of the extracts [16]. Table 1, summarizes some of the most recent research studies mentioned below.

In more detail, enzyme-assisted extraction (EAE) is widely reported by many scientific papers due to the appearance of many advantages such as the recovery of different classes of bioactive metabolites (e.g., polyphenols, carotenoids, polysaccharides, proteins, components of essential oil, and terpenes), the consideration as an eco-friendly and cost-effective improvement on classical or modern extraction methods. Łubek-Nguyen, et al. [17], in their study represent examples of studies on EAE of metabolites from natural materials such as plants, mushrooms, and animals mentioning

Table 1: Recent studies on extraction technologies.

Plant material, Extract, Bioactive ingredient	The extraction process, assays	Biological activity and use	Optimal extraction conditions	References
Pepper waste (<i>Capsicum annuum</i>)	Zinc oxide (ZnO) NPs	Antioxidant. Pepper used (whole fruit, pulp, or waste)	Temperature, extraction time, amount of pepper and solvent used 50 °C and 34.7 min	[16]
Plants, mushrooms, and animals	Enzyme-assisted extraction (EAE)	Nutraceutical and pharmaceutical application	Process optimization adapted to the material (enzyme type, particle size, hydration, etc.), metabolites, and enzyme used (e.g., temperature, concentration, pH, and duration)	[17]
Apple pomace	DPPH and the FRAP assay, deep eutectic solvent (DES) systems	Antioxidant	DES extracts from dry apple pomaces in ChCl: EG (1:4) resulted in high antioxidant results. Extraction times	[18]
Pomegranate peel, tannic acid, punicalin, punicalagin, ellagic acid		Antioxidant		[19,20]
Pomegranate peel	Vacuum Microwave-Assisted Aqueous Extraction (VMAAE)		Temperature, microwave power, time, and water/PP ratio. Extraction temperature (61.48 and 79.158 °C), time (10 and 12.17 min.), microwave power (3797.24 and 3576.37 W), the ratio of water to raw material (39/92% and 38.2%)	[21]
Pomegranate peel extracts		Antimicrobial		[22]
Pomegranate Peel	Simple Stirring, Pressure-Applied Extraction, Enzymatic Extraction, Ultrasound-Assisted Extraction, Pulsed Electric			[23]
Avocado fruit peels (AP) and seeds (AS)	VMAAE	Antioxidant	Temperature, time, the ratio of water to raw material, and microwave power. DPPH radical scavenging activity was equal to 100mg/L expressed to L of 0.104 l/min for AP and 0.045 l/min for AS	[24]
Apple peel		Antioxidant	Different CO ₂ concentrations (0-7.05 nmol/L) compared to different conventional solvents (distilled water and 10% ethanol/ethanol aqueous solvent) without and with ultrasound treatment at room temperature. The water CO ₂ system improves the extraction efficiency of polyphenols and active substances in apple peels using ultrasonication	[26]
Aronia and grapes	Ultrasound-Assisted Extraction		Different solid-to-solvent ratios and extraction time. At the 1:2 solid-to-solvent ratio, the color intensity, phenolics, and tannins content were improved using the ultrasound-assisted extraction of chokeberries	[27]
<i>E. nigrum</i> aerial parts	Ultrasound-Assisted Enzymatic Extraction (UAEE)	Antimicrobial	Temperature, incubation time. Incubation temperature of 38.39 °C, incubation time of 3.39 h, tannase of 386.53 U/g of the sample (U/g), and cellulase of 224.42 U/g)	[28]
Pomegranate peel, punicalagin	UAE-based extraction, separation, and purification methods		The yield of punicalagin increased when: Ethanol concentration increased from 40% to 50%, the sample-to-liquid ratio was in the range of 1:10–1:30 g/mL, ultrasonic power was at 500–800 W, and extraction time was within 20–40 min.	[29]
Red pitaya (<i>Hylocereus polyrhizus</i>) seeds	Subcritical Water Extraction (SWE) and three other conventional solvent extraction methods (CSE: water, ethanol, and acetone extraction)	Antioxidant	Optimal extraction conditions for subcritical water extraction of polyphenols from seeds were 15 min. reaction time at 220 °C with 2% solid-solvent ration (TPC: 63.14 mg GAE/g).	[30]

at the same time factors such as optimized conditions of enzymolysis and extraction effects and yields in optimal conditions. In the conclusion of their review, they highlighted the advantages of the EAE method.

The potential ability of a non-conventional green extraction method to extract bioactive compounds from apple pomace was studied by Bottu, et al. [18]. The authors examined the antioxidant capacity of extracts using the DPPH and the FRAP assay. From their results, it is suggested that deep eutectic solvent (DES) systems provide potential evidence and are promising options for the extraction of bioactive compounds from apple pomace that have relevance for metabolic health.

The pomegranate peel, a major processing by-product of pomegranate fruits, is an inexpensive and abundant source of polyphenols, mainly rich in ellagitannins, such as tannic acid, punicalin, punicalagin (which accounts for about 70% of the total ellagitannins), and ellagic acid [19,20]. Skenderidis, et al. [21], examined the optimization of the conditions applied for the extraction of pomegranate peels via a “green” industrial type of vacuum microwave-assisted aqueous extraction (VMAAE), by assessing the potential bioactivity of the extracts (in terms of phenolic content and antioxidant activity), using a response surface methodology. In their results, they concluded that the optimized industrial type of VMAAE could be a promising solution for the valorization of the PP by-products. Furthermore, Belgacem, et al. [22], in their review study highlighted the importance of pomegranate peel extract (PPE) and the complex mechanisms of action that include direct antimicrobial activity and induction of resistance in treated plant tissues, concluding that the broad spectrum of activity, the wide range of application and the high efficiency of PPE against bacterial, fungal and viral plant pathogens suggest a potential market not only restricted to organic production but also integrated farming systems. However, the quality characteristics of the bioactive compounds derived from pomegranate are dependent on the extraction method applied and the used solvent [23].

In a similar research study completed by Skenderidis, et al. [24], the VMAAE method was also used to maximize the extract's total phenols content (TPC), maximum antioxidant activity, and minimal operational cost of avocado fruit peels and seeds. According to the results presented in this research, VMAAE achieved the conversion of avocado by-product directly, without using previous cost-effective drying pre-treatment processes, into a valuable phytochemical ingredient for food, pharmaceutical, cosmetic and other industries, thereby providing a viable solution to avocado processing companies for the valorization of their by-products.

Apple peel also contains many nutrients, such as different polyphenols (chlorogenic acid, epicatechin, procyanidin B₂, phloretin, and quercetin) and vitamin C [25]. Thus, currently, the extraction of polyphenols from apple peels has attracted great attention, as apple peel is considered one of the potential sources of food antioxidants. Furthermore, Wang, et al. [26] studied the effect of extraction of polyphenols from apple peels using ultrasonication in water carbon dioxide (CO₂) systems

concluding that, the water CO₂ system could improve the extraction efficiency of polyphenols and active substances in apple peels using ultrasonication. More specifically, from the results of their study, it was obtained that the extraction TPC and water-holding capacity of apple peel samples both had a significant increase using ultrasonication for all solvents. In addition, the CO₂ concentration in the water CO₂ system of 5.28 mmol/L was optimal for polyphenols extraction, including total polyphenol content (TPC), total flavonoid content (TFC), and proanthocyanidins content and antioxidant capacity (DPPH) enhancement using ultrasonication.

The optimization of the ultrasound-assisted extraction of polyphenols from Aronia and grapes was the main subject of a research study completed by Watrelot and Bouska [27]. More specifically in their study, three solids-to-solvent ratios and times were applied on chokeberry and grape berries using 50% ethanol or 13% acidified ethanol and compared to a conventional extraction technique. From the results of their study, it is suggested that at the 1:2 solid-to-solvent ratio, the color intensity, phenolics, and tannins content were improved using the ultrasound-assisted extraction on chokeberries while the tannin content in grape berries remained the same.

Furthermore, in their study Gao, et al. [28] indicated that Ultrasound-Assisted Enzymatic Extraction (UAEE) was an effective technique for the extraction of total polyphenols (TPP) from the aerial parts of *E. nigrum*, an evergreen dwarf shrub of Empetraceae and TPP extracted by this extraction method could be applied in pharmaceuticals, cosmetics, and food industries. More specifically, under the optimum conditions, the TPP yield was 52.17 ± 0.39 mg/g, which was 1.62 and 1.73 times those of the methods of ultrasound-assisted extraction (UAE) and enzyme-assisted extraction (EAE), respectively. The purity of the crude extract purified with HPD-600 macroporous resin was 22.56%, which was 2.78 times higher than that of the crude extract. In addition, the results of antioxidant and antibacterial activity assays showed that the purified extract has stronger bioactivities in contrast with the crude extract.

Furthermore, Liu, et al. [29] explored pomegranate peel polyphenols using LC-MS/MS. In their study, they found that punicalagin was the most abundant compound. In more detail, the highest yield (505.89 ± 1.73 mg/g DW) of punicalagin was obtained by Ultrasonic-Assisted Extraction (UAE) with an ethanol concentration of 53%, the sample-to-liquid ratio of 1:25 w/v, ultrasonic power of 757 W, and extraction time of 25 min.

Shi, et al. [30] evaluated the optimal process for applying subcritical water extraction (SWE) to obtain polyphenols from red pitaya (*Hylocereus polyrhizus*) seeds, a native to Mexico but also widely cultivated all over the world, including in southern China and Malaysia, Thailand, Vietnam, and Australia exotic plant species [31]. Using Response Surface Methodology (RSM). According to the results presented in their study the optimal extraction conditions for subcritical water extraction of polyphenols from red pitaya seeds were 15 min reaction time at 220 °C with a 2% solid-solvent ratio (TPC: 63.14 mg GAE/g).

Bioactive compounds of plant species and wastes with potential use for different purposes

The availability of blueberry crop residues used in a sustainable circular bioeconomy was discussed in a review study completed by Liu, et al. [32]. Regarding bioeconomy, Srivastava, and Balakrishnan [33] mention in their study the importance of phytochemicals from fruit and vegetable wastes generated in hotels. According to their preliminary investigation, they revealed the presence of high TEAC values of various high-value compounds such as gallic acid, ferulic acid, rutin, and catechin in the phytochemicals extract obtained with both aqueous phase (ABTS) and organic phase (DPPH) assays. At this point, it is worthily to be mentioned that wet organic waste from the kitchen and gardens constitutes around 60–84% of the total waste generated [34–36] while waste from hotel kitchens is a mix of originally edible food waste and inedible biowastes [37] such as peels, seeds, and pulp from fruits and vegetables [35]. Thus, to improve the bioeconomy, there is significant potential to recover value-added phytochemicals from mixed fruit and vegetable wastes not only from houses but also from hotels. Table 2, represents some of the most recent studies regarding polyphenolic compounds and their activity on human health.

In more detail, the review of Liu, et al. [32] discusses the ample opportunities for strategic utilization of blueberry crop residues to offer solutions for environmental sustenance. It is well known that the antioxidant compounds and the dietary fibers from this particular residue are linked with several health benefits. The authors concluded that blueberry pomace could be used as a potential substrate for different purposes such as the production of value-added products which have large-scale applications mainly in food and pharmaceuticals, biofuel, etc., by reducing at the same time the ecological issues due to waste disposal. In another study, Najjar, et al. [38], reviewed the potential therapy of coronary microvascular dysfunction by highlighting the ability of berry-derived polyphenols concluding that further research is needed especially for future clinical trials in order to investigate the effectiveness of berries and their polyphenols in the treatment of coronary microvascular dysfunction (CMD) in Ischemia with no obstructive coronary artery disease (INOCA) patients.

Furthermore, Morton and Braakhuis [39] in their study indicated a protective effect on lung function from dietary intakes of fruit-derived polyphenols. However, they concluded that neither a benefit nor decrement from fruit-derived polyphenol intakes was detected for cognition, and thus, more conclusive results are needed to provide recommendations for polyphenol supplementation to support aspects of cognition. Finally, Wang, et al. [40] summarize the plant extracts and the possible mechanisms responsible for their anti-obesity effects while Rangarajan, et al. [41] reported the bioactive components and their potential health implications in chronic diseases of traditional fruits of South India.

The antioxidant activity of fruit and vegetable wastes confers health benefits such as protection from cardiovascular problems, cancers, and a variety of degenerative diseases [42]. Regarding the antioxidant activity of fruit extracts as antimicrobial agents against pathogenic bacteria, Suriyaprom, et al. [43] reported that various fruit extracts from citrus, berries, and pomegranates have been shown to possess a broad spectrum of medicinal properties. In their review study, it was also mentioned that fruit phytochemicals can directly inhibit bacterial growth or act indirectly by modulating the expression of virulence factors, both of which reduce microbial pathogenicity. In order to support these findings, they listed various fruit extracts and their major bioactive compounds, determining the effectiveness of organic acids, terpenes, polyphenols, and other types of phenolic compounds with antioxidant properties as a source of antimicrobial agents. Promoting this antioxidant activity Kurek, et al. [14] in their review paper gave an insight into the effective delivery mechanisms for health-promoting substances and highlights the challenges of using antioxidants and bioactive ingredients in foods.

Arfaoui, et al. [44] represent an overview of studies investigating the food-processing effects on polyphenols' content and antioxidant activity. The same author also, critically reviewed the status of knowledge on the possible (positive or negative) effects of commonly used food-processing techniques on phenolic compound content and bioavailability in fruits and vegetables and the importance of more comprehensive dietary

Table 2: Recent studies on health promotion by bioactive ingredients.

Source of bioactive ingredients	Bioactivity and use	References
Blueberry crop residues	Production of different value-added compounds	[32]
Different berries	Coronary microvascular dysfunction (CMD) in Ischemia	[38]
Fruit polyphenols	Protective effect on lung function	[39]
Extracts from plants, such as vegetables, tea, fruits, and Chinese herbal medicine	Anti-obesity effects	[40]
Traditional fruits of South India	Various health complications such as diabetes, obesity, cancer, cardiovascular disease, immune system decline, and certain neurological disorders	[41]
Citrus, berries, and pomegranates	Decreases the development of bacterial resistance and preserves probiotic species in the microbiota	[43]
Several fruits and vegetables	Antioxidant activity	[44]
Several vegetables, grape pomace, coffee	Promoting growth and differentiation of osteoblasts	[45]
<i>Pleioblastus amarus</i> (<i>P. amarus</i>) shoots	Antioxidant, anti-inflammatory activity	[46]
Rice and corn bran	Improved <i>in vitro</i> digestion, anti-inflammatory responses, and overall quality	[54]

guidelines that consider the recommendations of processing parameters to take full advantage of phenolic compounds toward healthier foods.

Recent advances in food and health applications of natural phenol polymers were reviewed by Panzella and Napolitano [45]. Several *in vivo* human and animal trials over the last few years supported that natural phenol-based polymers appear beneficial effects and can be used in regenerative biomedicine as additives of biomaterials to promote the growth and differentiation of osteoblasts. Furthermore, other studies mentioned the innovative application of natural phenol polymers such as condensed tannins as animal feed and food supplements, hydrolyzable tannins as food supplements and in food packaging, while, bio-inspired phenolic polymers and lignins can be used also in food packaging, and phlorotannins can be used in tissue engineering.

Ao, et al. [46] studied the phenolic compounds of a traditional green vegetable in China belonging to the grass family Gramineae the *Pleioblastus amarus*. According to their study shoots of this plant species are rich in nutritional properties, and can provide various health benefits [47–50].

Finally, despite fruits and vegetables also seed grains and by-products of cereals are considered good sources of bioactive compounds such as protein, fiber, and micronutrients like phenolic compounds, vitamins, and minerals [51,52]. However, around 69 and 74% of the total phenolics present in corn and rice, respectively, are in insoluble form [52,53]. Mehta, et al. [54] in their study investigated the impact of atmospheric and vacuum cold plasma on the extraction efficiency of polyphenols from de-oiled rice and corn bran. From the results presented in their study, it was mentioned that cold plasma processing was observed to significantly enhance the content of individual polyphenols extracted from rice bran (vanillin, ferulic acid, sinapic acid, and chlorogenic acid) and corn bran (4-hydroxybenzaldehyde, p-coumaric, sinapic acid and ferulic acid) in comparison with conventional extraction while significant increment was also found in total phenolic content, total flavonoid content and antioxidant activity of extracted polyphenols.

Conclusion

Most plant species produce a broad range of secondary metabolites in very low quantities with their production mainly depending on the development and physiological stage of the plants. These secondary metabolites not only play important role in plant-microbe interactions but are also used by humans in modern and traditional industries as medicines, flavorings, pharmaceuticals, agrochemicals, fragrances, colors, biopesticides, food additives, and drugs. Thus, the recovery of high-added-value products from waste plant material is a significant issue and phytochemical investigation aims to obtain new naturally active substances to act as an antioxidant, promoting human health. However, limitations in the efficient extraction of these bioactive substances have drawn the attention of the scientific community worldwide and so far, different extraction methods have been proposed for the efficient extraction of several bioactive compounds.

Author contributions

Conceptualization, S.L., and P.S.; investigation, P.S.; writing—original draft preparation, S.L., and VC.; writing—review and editing, S.L. and VC.; visualization, S.L., and P.S.; supervision, P.S. All authors have read and agreed to the published version of this manuscript.

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