Recent trends in extraction, purification, and antioxidant activity evaluation of plant leaf-extract polysaccharides

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Received April 30 2022; Revised June 08 2022; Accepted June 27 2022;
View online July 29, 2022 at Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.2405; Biofuels, Bioprod. Bioref. 16:1820–1848 (2022)

Abstract: This review elaborates on current advances in the extraction, purification, and antioxidant activity of plant leaf-extract polysaccharides. Polysaccharides are widely used as important ingredients in the food, pharmaceutical, and cosmetic industries. Researchers have been investigating useful sources of natural polysaccharides and developing green and feasible extraction procedures for polysaccharides. This review examines different methods for extracting polysaccharides from leaves, and discusses their advantages and limitations. Purification techniques for plant leaf-based polysaccharides were also highlighted, together with their antioxidant effects. Among different extraction methods, pressurized-liquid extraction and enzyme-assisted extraction are considered to be better for large-scale extraction of polysaccharides from plant leaves. This review could contribute to the design of leaf waste processes at a commercial level for the sustainable recovery of polysaccharides. © 2022 The Authors. Biofuels, Bioproducts and Biorefining published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Key words: polysaccharides; plant leaves; extraction; purification; antioxidant activities

Introduction

Polysaccharides have many biological functions and many applications. Natural polysaccharides originate from plants, animals, and microorganisms. Polysaccharides extracted from plant leaves exhibit significant differences in structure and processibility. Generally, in polysaccharides, monomers (usually >10) are covalently linked either in the form of linear or branched glucosidic linkage. The extraction of polysaccharides from plant leaves with a high yield is important for their economic application. Several extraction techniques have been established for the
preparation of polysaccharides but the selection of the one with best commercial feasibility is important to manage leaf waste. The chemical composition and structure of polysaccharides act as key aspects to characterize, and the understanding of these is important for tailor-made applications. Analytical techniques and chemical methods have been developed for the extraction and purification of polysaccharides, and an understanding of their advantages and limitations supports their use for commercial purposes.

The polysaccharides have now been broadly exploited in the medicinal, food, textile, cosmetics, leather-tanning, electronics, and mechanical industries due to their wide availability, low cost, biodegradability, non-toxic nature, renewability, environment-friendly behavior, and multiple biological functions with negligible side effects. Previous studies have demonstrated that polysaccharides can be used as active agents in medicines due to their biological activity – for example their antioxidant, anti-tumor, anticoagulant, anti-virus, anti-radiation, anti-cancer, and immunoregulatory activity. The broad range of medicinal functions of polysaccharides and their antioxidant activity have attracted wide attention due to their feasible transfer of value-added properties to the entire system with negligible disturbance.

In other words, the vigorous role of polysaccharides as modifiers of biological systems and to inhibit the potential of oxidative stress by scavenging the free radicals cannot be ignored. The potential application and antioxidant activity of polysaccharides also depend on the structure of the molecules and functional groups.

Plant biomass is loaded with useful phytochemicals and biomolecules. Plant biomass commonly consists of plant leaves, branches, bark, grass, flowers, fruits, and other woody materials. The leaves of plants account for the largest portion of plant biomass. In autumn, a large amount of plant leaves drop onto the ground surface, which increases the volume of plant biomass. Plant biomass has been widely used for the removal of heavy metals, phenols, dyes, and other organic pollutants from wastewater through adsorption processes for water purification.

Consumption of plant biomass for adsorption purposes is not a sustainable solution for biomass management because the adsorption process generates pollutant-loaded biomass, which is more difficult to manage than the initial one. Previous studies suggest that the waste generated by plants can be managed by utilizing aerobic and anaerobic digestion techniques, but these technologies require significant investment for equipment, create several environmental issues, and might not be economically feasible on a large scale. The production of biofuel, bioenergy, bioplastic, and biogas can be other possible routes for the conversion of plant biomass into value-added products but a large financial investment is required to commercialize these possibilities. There is thus a need to provide alternative cost-effective, easy, and feasible solutions to manage plant biomass on a commercial scale. Given these challenges, this review was compiled to discuss common methods for the extraction and purification of polysaccharides and resultant antioxidant activity, to manage plant leaves on an industrial scale.

Several reviews have been published on the extraction of polysaccharides from plants, seaweeds, microbes, and other natural sources, with the biological potential for polysaccharides highlighted. However, these reviews did not discuss the processing of polysaccharides on a large scale. The present review is therefore designed to compare different extraction methods for polysaccharides with large-scale schematic layouts and to suggest the most promising ones. The methods of purifying these polysaccharides, their antioxidant activity, and their large-scale applications are also highlighted in this review.

### Extraction methods for polysaccharides

Researchers are focusing on developing a bundle of extraction procedures to prepare polysaccharides without disturbing their structural composition. Polysaccharides from natural sources (e.g. plants, animals, and microbes) have been extracted successfully using several extraction techniques. Although all these extraction techniques, which are described below, can be used for the extraction of polysaccharides from different sources, the data presented in this review are to provide insights into the utilization of leaf-extract polysaccharides. In this section, different methods of polysaccharide extraction are discussed based on mechanisms and operating conditions. These methods of extracting polysaccharides are depicted in Figure 1.

### Commonly used methods

Extraction methods commonly used for polysaccharides include hot-water extraction (HWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized-liquid extraction (PLE), supercritical-fluid extraction (SFE), and enzyme-assisted extraction (EAE).

#### Hot-water extraction

Hot-water extraction (HWE) is an easy traditional method for the extraction of polysaccharides. In this method, water is heated up to 100 °C, and the extraction tank is loaded with plant material at a certain material-to-water ratio; then, the extraction is carried out under a controlled temperature for...
a specified duration and polysaccharide-containing extracts are obtained by a jacketed vessel where the extract is cooled and separated from plant material (Figure 2). Extraction time, temperature, and material-to-water ratio are key factors that affect the extraction efficiency of polysaccharides.\textsuperscript{26} For the optimization of these factors, mathematical designs such as continuous single-factor and orthogonal designs and statistical designs like response surface with Box–Behnken designs (BBD) or central composite designs (CCD) can be used.

In HWE, a high temperature is required to extract plant polysaccharides. Generally, at high temperature, Maillard reaction and caramelization may occur, which degrades polysaccharides and leads to poor extraction efficiency.\textsuperscript{6} At high temperatures, some of the reducing sugars may react with amino acids present in plant materials to produce complex compounds, which may interact with polysaccharides and cause them to degrade and thus reduce the extraction yield. Generally, in HWE, a material-to-liquid ratio of about 1:20 is used to concentrate the extract, and
thermal degradation of polysaccharides can occur. Other disadvantages of this method are a long extraction time and a large volume of ethanol (4–10 times) required to precipitate crude polysaccharides. Previous studies suggested that HWE can only extract extracellular polysaccharides and cannot destroy the cell wall and plasma membrane of plant materials. Polysaccharides from *Hizophora mucronate* leaves showed a 7.67% extraction yield when being processed using an HWE system at a solid-to-liquid ratio of 1/50 (w/v) and a temperature of 90 °C for 3 h. Hot-water extraction was applied to extract polysaccharides from *Aconitum carmichaelii* leaves, which showed a 4.2% extraction yield when the extraction was carried out at a solid-to-liquid ratio of 1:20 (w/v) and a temperature of above 90 °C (under reflux) with a 1 h extraction time twice. Polysaccharides extracted from *Tymus quinquecostatus* leaves showed an 86% extraction yield when being processed at a solid-to-liquid ratio of 1:20 (w/v) and a temperature of 100 °C for 3 h using HWE. Many other studies have involved polysaccharide extraction using HWE from plant leaves as listed in Table 1.

### Microwave-assisted extraction

The microwave-assisted extraction (MAE) method can be used for polysaccharide extraction due to its strong penetration, high selectivity, and high efficiency. In an electromagnetic field, microwaves with non-ionizing radiation within the energy range 300 MHz to 300 GHz penetrate plant material and generate volumetrically distributed heat through molecular friction. Microwaves break the cell wall and inactivate enzymes in the cell membrane to extract polysaccharides. In MAE, microwave and electromagnetic radiation passed through the extraction chamber, which contains water and plant material, leading to an increased temperature, the destruction of the cell wall and cell membrane, and the increased polarization of polysaccharide molecules (Fig. 3). After extraction, the resultant slurry passed through a jacketed vessel where the extract was separated from plant material and cooled to room temperature. Remarkably, MAE possesses some advantages over other conventional methods, such as being financially feasible, time saving, and eco-friendly, and possessing high extraction efficiency with the minimal use of solvents, and low energy consumption. The glycosidic linkages in polysaccharides can be disturbed by intense microwave treatment for a long time. High microwave power and intense electromagnetic radiation also depolymerize polysaccharides chains. Hence, the microwave power and extraction time should be controlled stringently to prevent polysaccharide degradation. A high temperature in MAE also reduces the extraction yield because, at a high temperature, a browning reaction (caramelization) takes place. Polysaccharides from *Eucommia ulmoides* leaves were extracted using MAE and showed a 12.31% extraction yield when being processed at a solid-to-liquid ratio of 1:29 (w/v) and a temperature of 74 °C with a reaction time of 15 min. Pectin from *Premna microphylla* leaves extracted using MAE showed an 18.25% extraction yield when the extraction was carried out at a solid-to-liquid ratio of 1:50 (w/v), a temperature of 90 °C, and a pH of 2 for 2 h. Different polysaccharides extracted from leaves using MAE are listed in Table 1.

### Ultrasound-assisted extraction

In UAE, ultrasonic waves rupture plant cells, thus leading to polysaccharide extraction. Ultrasound waves generated from the probe travel to the medium (water) and produce cavitation bubbles, which strike plant cells in the extraction chamber and destroy plant cells at a certain temperature, leading to polysaccharide extraction (Fig. 4). After extraction, the whole extract, including plant material, pass through a jacketed vessel where the extract is separated from plant material and cool to room temperature. Ultrasound waves with a power level of 10–100 kHz are normally used to generate cavitation bubbles, and the cavitation process makes polysaccharides diffuse from the cell wall, which can increase extraction efficiency significantly.

In UAE, a higher temperature increases the kinetic energy of gas-phase cavitation bubbles, which weakens the cell-wall polysaccharides, thus leading to high extraction efficiency. A great advantage of ultrasonic extraction is that it is fast. The extraction efficiency of polysaccharides by using ultrasonic extraction is affected by extraction time, ultrasonic power, and temperature. When high ultrasound power is applied for long periods, a decrease in extraction efficiency was observed due to the destruction of glycosidic linkages and depolymerization. To overcome these drawbacks, it is necessary to optimize parameters such as extraction time, ultrasound power, and temperature. Ultrasound-assisted extraction was applied to polysaccharides from *Sorghum bicolor* leaves and showed a 9.23% extraction yield at a solid-to-liquid ratio of 1:20 (w/v), an ultrasound frequency of 60 kHz, an ultrasound power of 240 W, and a temperature of 70 °C, with an extraction time of 70 min. Some other leaf-extract polysaccharides using UAE are listed in Table 1.

### Pressurized-liquid extraction

In pressurized-liquid extraction (PLE), elevated temperature and high pressure are employed in automated extraction media to keep the solvent in the liquid phase, and these conditions play a meaningful role in increasing...
### Table 1. Extraction and purification of plant leaf-extract polysaccharides

| Leaves              | Extraction method | Extraction conditions                        | Purification                        | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|---------------------|-------------------|---------------------------------------------|-------------------------------------|---------------|-----------|------------------------|-----------|
| Tonna sinensis      | HWE               | Solid-to-liquid ratio, 1:30 w/v, temp. 80°C, time, 2 h | DEAE cellulose anion exchange, chromatography, Sephacryl S-400-GPC | TSP-1         | 33.4      | 833.6                  | 31        |
|                     |                   |                                             |                                     | TSP-2         | 26.6      | 81.6                   |           |
| Eriobotrya japonica | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 95°C, time, 2 h | HPSEC with MALLS-RID | LLP-B         | 3.62      | 5101                   | 32        |
|                     |                   |                                             |                                     | LLP-C         | 3.95      | 4786                   |           |
|                     |                   |                                             |                                     | LLP-D         | 5.29      | 4307                   |           |
|                     |                   |                                             |                                     | LLP-Y         | 3.94      | 4605                   |           |
| Nelumbo nucifera    | HWE               | Solid-to-liquid ratio, 1:20 w/v, time, 2 h under reflux | Sephacryl G-100 GPC | LLWP-1        | 19.9      | 85.1                   | 33        |
|                     |                   |                                             |                                     | LLWP-3        | 21.3      | 12.5                   |           |
| Ginkgo biloba       | HWE               | Solid-to-liquid ratio, 1:10 w/v, temp. 70°C, time, 3 h | DEAE Sepharose column chromatography | GBPS-2        | 9.45      | 672                    | 34        |
|                     |                   |                                             |                                     | GBPS-3        | 32.87     | 723                    |           |
| Moringa oleifera    | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time, 4 h | DEAE Sepharose ion exchange chromatography | MOP-2        | 6.84      | 155.35                | 35        |
| Olive               | HWE               | Solid-to-liquid ratio, 1:30 w/v, temp. 90°C, time, 4 h | ND | OLP         | 7.2       | ND                     | 36        |
| Plantago ovata      | HWE               | Solid-to-liquid ratio, 1:50 w/v, temp. 90°C, time, 4 h | DEAE cellulose column chromatography | W            | ND        | 10.7                   | 37        |
|                     |                   |                                             |                                     | W2           | 13        | 18.2                   |           |
|                     |                   |                                             |                                     | W3           | 1         | 60.6                   |           |
| Lepidium meyenii    | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time, 3 h | DEAE-52 cellulose column chromatography, Sephadex G-200 column chromatography | MLP-1        | 35.7      | 42.756, 93.541         | 38        |
|                     |                   |                                             |                                     | MLP-2        | 25.3      |                         |           |
| Acanthopanax        | HWE               | Solid-to-liquid ratio, 3:5 w/v, temp. 90°C, time, 3 h | Sephacryl S-100 column chromatography | ASP-B2        | ND        | 5.32                   | 39        |
|                     |                   |                                             |                                     | ASP-B3        | ND        | 30.51                  |           |
| Lipidium meyenii    | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time, 2 h | DEAE-52 cellulose and Sephadex-100 column chromatography | LMLP         | ND        | 58.43                  | 40        |
| Lilium lancifolium  | HWE               | Solid-to-liquid ratio, ND, temp. 70°C, time 1 h | DEAE-52 cellulose column chromatography | LLP-1        | 20.13     | 2250                   | 41        |
|                     |                   |                                             |                                     | LLP-2        | 13.07     | 2020                   |           |
|                     |                   |                                             |                                     | LLP-3        | 9.85      | 2080                   |           |
| Arctium lappa       | HWE               | Solid-to-liquid ratio, 1:20 w/v, under reflux, time 2 h | Ultrafiltration using cellulose Easter membrane | SAA          | 13.19     | ND                     | 42        |
|                     |                   |                                             |                                     | RF50          | 24.7      | ND                     |           |
|                     |                   |                                             |                                     | RF30          | 16.01     | ND                     |           |
|                     |                   |                                             |                                     | EF30          | 59.3      | ND                     |           |
| Morus alba L.       | HWE               | Solid-to-liquid ratio, 1:34 w/v, temp. 92°C, time 3.5 h | DEAE-52 cellulose and Sephadex G-100 column chromatography | MLP          | 10.00     | ND                     | 43        |
|                     |                   |                                             |                                     | MLP-3a        | ND        | 80.99                  |           |
|                     |                   |                                             |                                     | MLP-3b        | ND        | 3.64                   |           |
| Paris polyphylla    | HWE               | Solid-to-liquid ratio, 1:21.3 w/v, temp. 90.8°C, time 4 h | DEAE cellulose column chromatography | PPLPs         | 54.18     | ND                     | 44        |

(Continued)
| Leaves                        | Extraction method | Extraction conditions                        | Purification                          | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|------------------------------|-------------------|---------------------------------------------|---------------------------------------|---------------|------------|------------------------|-----------|
| Cyclocarya paliurus          | HWE               | Solid-to-liquid ratio, ND, temp. 80°C, time 3 h | ND                                    | CP            | ND         | ND                     | 1050-1090 | 45        |
|                              |                   |                                             |                                       | Ac-CP1        | 68.78      |                        |           |
|                              |                   |                                             |                                       | Ac-CP2        | 73.12      |                        |           |
|                              |                   |                                             |                                       | Ac-CP3        | 86.75      |                        |           |
| Leonurus cardiaca            | HWE               | Solid-to-liquid ratio, 1:45.2 w/v, temp. 81.4°C, time 1.7 h | ND                                    | LCLP          | 9.17       | ND                     |           |
|                              |                   |                                             |                                       |               |            |                        | 70-110    | 46        |
| Cyclocarya paliurus          | HWE               | Solid-to-liquid ratio, 1:40 w/v, temp. 80°C, time 3 h | Anion exchange chromatography         | CPP           | 2.16       | 900                    | 16        |
| Morus alba                   | HWE               | Solid-to-liquid ratio, 1:40 w/v, temp. 85°C, time 5 h | ND                                    | MLCP          | 12.01      | ND                     |           |
| Sambucus adanata             | HWE               | Solid-to-liquid ratio, 1:26 w/v, temp. 89°C, time 14 min | DEAE-Sepharose fast flow column chromatography | SPW-2         | ND         | 7.04                   |           |
| Mulberry                     | HWE               | Solid-to-liquid ratio, 1:28.91 w/v, temp. 86.32°C, time 1 h | Dynamic adsorption using activated carbon microporous resins | MLP-80        | 18.64      | ND                     |           |
| Camellia sinensis            | HWE; EAE          | Solid-to-liquid ratio, 1:40 w/v, temp. 90°C, time 2 h; Solid-to-liquid ratio, 1:40 w/v, temp. 45°C, pH 5.5, time 2 h | ND; | HWE-TLPs     | 1.28       | 1.165-412               |           |
|                              |                   |                                             |                                       | EAE-TLPs      | 4.08       | 75.9-487                |           |
|                              |                   |                                             |                                       |               |            |                        | 110-150   | 50        |
| Cyclocarya paliurus          | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 80°C, time 2 h | ND                                    | CP            | ND         | ND                     |           |
|                              |                   |                                             |                                       | CM-CP1        | 58.78      | 1030                   |           |
|                              |                   |                                             |                                       | CM-CP2        | 36.88      | 1050                   |           |
|                              |                   |                                             |                                       | CM-CP3        | 78.42      | 1080                   |           |
| Handroanthus heptaphyllus    | HWE               | Solid-to-liquid ratio, 2:5 w/v, time 2 h, under reflux | ND                                    | HHSF          | 3.7        | 9.4                    |           |
| Lycium barbarum             | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time 1 h | DEAE-Sephadex A25 column chromatography | CBP           | 16.21      | ND                     |           |
|                              |                   |                                             |                                       | CBP-II        | ND         | 93.9                   |           |
|                              |                   |                                             |                                       | CBP-IV        | ND         | 418                    |           |
| Catharanthus roseus          | HWE               | Solid-to-liquid ratio, ND, temp. 100°C, time 6 h | Ultradifiltration; using cellulose membrane | PS-1          | 32         | 7.4                    |           |
|                              |                   |                                             |                                       |               | 200        |                        |           |
| Ilex latifolia               | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time 2 h | DEAE-52 cellulose column chromatography | ILPS          | 6.3        | ND                     |           |
|                              |                   |                                             |                                       | ILPS-1        | 32.3       |                        |           |
|                              |                   |                                             |                                       | ILPS-2        | 20.6       |                        |           |
|                              |                   |                                             |                                       | ILPS-3        | 18.4       |                        |           |
|                              |                   |                                             |                                       | ILPS-4        | 10.8       |                        |           |
| Clinacanthus nutans         | HWE               | Solid-to-liquid ratio, 1:6 w/v, temp. 90°C, time 4 h | Superdex 200 and DEAE Sepharose fast flow column chromatography | CNP1-2        | ND         | 91.7                   |           |
|                              |                   |                                             |                                       |               |            |                        | 110-150   | 56        |
| Leaves                        | Extraction method | Extraction conditions                              | Purification                                                                 | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|------------------------------|-------------------|---------------------------------------------------|------------------------------------------------------------------------------|---------------|-----------|------------------------|-----------|
| Alchornea cordifolia         | HWE               | Solid-to-liquid ratio, 1:60 w/v, temp. 100°C, time 1 h | DEAE cellulose, Dianion HP-20 and Sepharose 6B column chromatography          | AP-AB         | ND        | 8.9                    | 62        |
|                              |                   |                                                   |                                                                               | AP-AU         | 6.6       |                        |           |
|                              |                   |                                                   |                                                                               | AP-AU1        | 39.5      |                        |           |
|                              |                   |                                                   |                                                                               | AP-NU         | 4.9       |                        |           |
| Gynura procumbens            | HWE               | Solid-to-liquid ratio, 1:50 w/v, temp. 100°C, time 3 h | Fractional precipitation using different concentrations of ethanol           | GPP-20        | 14.00     | ND                     | 58        |
|                              |                   |                                                   |                                                                               | GPP-40        | 32.80     |                        |           |
|                              |                   |                                                   |                                                                               | GPP-60        | 26.43     |                        |           |
|                              |                   |                                                   |                                                                               | GPP-80        | 7.96      |                        |           |
| Aralia elate                 | HWE               | Solid-to-liquid ratio, ND, temp. 90°C, time 3 h    | DEAE-52 cellulose and Sephadex G-100 column chromatography                    | AEC-1         | 2.27      | ND                     | 59        |
| Moso bamboo                  | HWE               | Solid-to-liquid ratio, ND, temp. 100°C, time 2 h   | DEAE Sepharose fast flow column chromatography                               | WB1           | ND        | 134                    | 60        |
| Arthocnemum indicum          | HWE               | Solid-to-liquid ratio, 1:40 w/v, temp. 80°C, time 4 h | ND                                                                           | PAI           | 19.7      | ND                     | 61        |
| Bruguiera gymnorrhiza        | HWE               | Solid-to-liquid ratio, 1:42 w/v, temp. 71°C, time 0.5 h | ND                                                                           | BGP            | 16.43     | ND                     | 57        |
| Malva sylvestris             | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 90°C, time 1 h | ND                                                                           | MSLP          | 8.37      | ND                     | 63        |
| Cyclocarya paliurus          | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 80°C, time 2 h | Dialysis method                                                              | CP            | ND        | ND                     | 1         |
|                              |                   |                                                   |                                                                               | S-CP1-1       | 118.85    | ND                     |           |
| Paris polyphylla             | HWE               | Solid-to-liquid ratio, 1:21.3 w/v, temp. 90.8°C, 4.8 h | DEAE-52 cellulose column chromatography                                      | PPLPC         | ND        | 29.5                   | 64        |
| Hoheria populnea             | HWE               | Solid-to-liquid ratio, ND, temp. 65°C, time 2 h    | Ultrifiltration                                                             | ND            | 1.7       | 2310                   | 65        |
| Tiliacora triandra           | HWE               | Solid-to-liquid ratio, 1:6.6 w/v, temp. 85°C, time 1.67 h | ND                                                                           | Yanang gum    | 0.8       | ND                     | 66        |
| Althaea officinalis          | HWE               | Solid-to-liquid ratio, 1:39.1, temp. 83.1°C, time 1.16 h | DEAE-52 cellulose column chromatography                                     | AOL-1         | 14.47     | ND                     | 67        |
|                              |                   |                                                   |                                                                               | AOL-2         | 1.220     |                        |           |
| Taxus yunnanensis            | HWE               | Solid-to-liquid ratio, 3:40 w/v, temp. 85-90°C, time 8 h | DEAE-52 cellulose column chromatography                                    | TMP70W        | 1.9       | 36.94                   | 68        |
| Leaves                | Extraction method | Extraction conditions                                      | Purification                                         | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|-----------------------|-------------------|----------------------------------------------------------|------------------------------------------------------|---------------|-----------|------------------------|-----------|
| Premna microphylla    | HWE               | Solid-to-liquid ratio, 1:50 w/v, temp 90°C, time 2 h, pH 2.0 | ND                                                   | PML           | 18.25     | 2.65, 18.35            | 69        |
| Azadirachta indica    | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 20-30°C, time 6 h  | Dialysis method                                       | PI            | 0.85      | 80                     | 70        |
| Ginkgo biloba         | HWE               | Solid-to-liquid ratio, 1:30 w/v, temp. 80°C, time 6 h    | Sephadex G-75 column chromatography                   | GBLP          | 4.28      | 12                     | 71        |
| Cyclocarya paliurus   | HWE               | Solid-to-liquid ratio, 1:20 w/v, temp. 80°C, time 2 h    | DEAE Sephadex A25 and Sephacryl S400 column chromatography | CPP, CPP-1, CPP2 | 4.56, 76.6 | ND, 1167                | 72        |
| Moringa oleifera      | MAE               | Solid-to-liquid ratio, 1:35 w/v, temp. 70°C, time 1.17 h, microwave power 700 W | Sequential precipitation using different concentrations of ethanol | MLP, MLP-1, MLP-2, MLP-3 | 2.96, ND | ND, 6290, 4860       | 73        |
| Mulberry              | MAE               | Solid-to-liquid ratio, 1:5 w/v, time 0.167 h, microwave power 170 W, | ND                                                   | MLP           | 9.41      | ND                     | 74        |
| Phyllostachys pubescens | UAE         | Solid-to-liquid ratio, 1:20 w/v, time 1.5h, ultrasound power 50 W | DEAE Sepharose fast flow column chromatography       | NPs, NPs-A, NPs-B | 10.2, 9.2 | 5.77, 4.30            | 75        |
| Guava                 | UAE               | Solid-to-liquid ratio, 1:10 w/v, temp. 62°C, time 0.33 h, ultrasound power 404 W | ND                                                   | GLP           | 1         | ND                     | 76        |
| Suaeda fruticosa      | UAE               | Solid-to-liquid ratio, ND, temp. 90°C, time 0.58 h, pH 2.9, ultrasound frequency 50kHz | Dialysis method                                       | SFP           | 34        | 240                    | 77        |
| Cyclocarya paliurus   | UAE               | Solid-to-liquid ratio, 1:10 w/v, temp.80°C               | DEAE cellulose column chromatography                  | CPP, CPP-D   | ND        | 1150, 9.1              | 78        |
| Isodon Lophantheoides | UAE               | Solid-to-liquid ratio, 1:17.5 w/v, temp. ND, time 74.74 min, ultrasound power 450 W, pH 8.5 | Sequential precipitation using different concentrations of ethanol | ILHP, ILHP-3 | 77.50, ND | ND, 247               | 79        |
| Hibiscus              | UAE               | Solid-to-liquid ratio, 1:24.31 w/v, temp. 83.18°C, time 0.42 h, ultrasound power; 93.59 W | ND                                                   | HRLP          | 9.66      | ND                     | 80        |
| Leaves                     | Extraction method | Extraction conditions                                                                 | Purification                                      | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|---------------------------|-------------------|----------------------------------------------------------------------------------------|---------------------------------------------------|---------------|-----------|------------------------|-----------|
| Epimedium                 | UAE               | Solid-to-liquid ratio, ND, temp. 46.8°C; time 0.71 h, pH 4.28, ultrasound power 311 W | DEAE Sepharose and Sephadex G-100 column chromatography | CEP           | 5.98      | ND                     | 81        |
|                           |                   |                                                                                        |                                                   | EP-1          | 20.86     | 81.64                  |           |
|                           |                   |                                                                                        |                                                   | EP-2          | 26.02     | 60.53                  |           |
|                           |                   |                                                                                        |                                                   | EP-3          | 14.52     | 21.85                  |           |
| Mentha haplocalyx         | UAE               | Solid-to-liquid ratio, 1:29 w/v, temp. 70°C, time 0.47 h, ultrasound power 300 W       | Dialysis method                                   | MHP           | 9.41      | 59.58                  | 82        |
| Camellia oleifera         | UAE               | Solid-to-liquid ratio, 1:20 w/v, temp. 88°C, time 1.6 h, ultrasound frequency 45kHz   | DEAE cellulose column chromatography              | CLP           | 3.77      | ND                     | 83        |
|                           |                   |                                                                                        |                                                   | CLP-1         | ND        | 78.954                 |           |
|                           |                   |                                                                                        |                                                   | CLP-2         | ND        | 51.257                 |           |
|                           |                   |                                                                                        |                                                   | CLP-3         | ND        | 60.143                 |           |
| Rhododendron aganniphum   | UAE               | Solid-to-liquid ratio, 1:25 w/v, temp. 55°C, time 2.2 h, ultrasound power 200 W       | ND                                                | ND            | 9.428     | ND                     | 84        |
| Dodonaea viscosa          | UAE               | Solid-to-liquid ratio, ND, temp. 85°C, time 0.84 h, ultrasound power 400 W            | ND                                                | ND            | 6.455     | ND                     | 85        |
| Quercus brantii           | UAE               | Solid-to-liquid ratio, 1:23.4 w/v, temp. 81.9°C, time 0.93 h, ultrasound power 205.87 W | ND                                                | QBLP          | 19.42     | ND                     | 86        |
| Phyllostachys Heterocycle | SFE               | Extraction time 2 h, temp. 50°C, pressure of CO₂ 40 MPa, modifier dosage 30 mL       | DEAE cellulose column chromatography              | BLPs          | 2.47      | 50.5                   | 87        |
| Ginkgo biloba             | UAE               | Solid-to-liquid ratio, ND, temp. 51.88°C, time 0.62 h, pH 4.34                         | ND                                                | GBLP          | 7.29      | ND                     | 88        |
| Lotus                     | EAE; HWE          | Solid-to-liquid ratio, 1:20 w/v, temp. 50°C, time 48 h, pH 4.5–5.0, enzymes (protease, amylase, pectinase, cellulase); Solid-to-liquid ratio, 1:20 w/v, temp. 100°C, time 4 h | Ultrafiltration and Sephadex G-100 column chromatography | LLEP-P-1      | ND        | 14.63                  | 89        |
|                           |                   |                                                                                        |                                                   | LLWP          | 1.18      | ND                     |           |
|                           |                   |                                                                                        |                                                   | LLEP-A        | 0.97      | ND                     |           |
|                           |                   |                                                                                        |                                                   | LLEP-C        | 1.17      | ND                     |           |
|                           |                   |                                                                                        |                                                   | LLEP-P        | 1.11      | ND                     |           |
|                           |                   |                                                                                        |                                                   | LLEP-PR       | 1.93      | ND                     |           |
| Silphium Perfoliatum      | EAE               | Solid-to-liquid ratio, 1:22 w/v, enzyme complex having concentration 1.59%             | DEAE-52 cellulose and Sepharose CL-6B column chromatography | CPP           | 13.69     | ND                     | 90        |
|                           |                   |                                                                                        |                                                   | CPP1-2        | ND        | 11.733                 |           |

(Continued)
| Leaves                        | Extraction method | Extraction conditions                                                                 | Purification       | Compound name | Yield (%) | Molecular weight (kDa) | Reference |
|------------------------------|-------------------|---------------------------------------------------------------------------------------|--------------------|---------------|-----------|------------------------|-----------|
| *Viscum Coloratum*           | EAE               | Solid-to-liquid ratio, 1:40 w/v, temp. 50°C, time 0.67, pH 5, enzyme concentration 2.5% | ND                 | VCP           | 21.83     | ND                     | 91        |
| *Malva sylvestris*           | EAE               | Solid-to-liquid ratio, ND, temp. 55.65°C, time 3.4h, pH 5.22, cellulase concentration 5.64% | DEAE cellulose and Sephadex G-100 column chromatography | MSPs          | 10.4      | 2600-8800              | 92        |
| *axus cuspidate*             | EAE               | Solid-to-liquid ratio, 1:19 w/v, temp. 51°C, time 33 min, enzyme dosage 0.10mg/mL       | DEAE cellulose column chromatography | MSP-1         | ND        | ND                     | 93        |
| *Sagittaria Sagittifolia*    | SWE               | Solid-to-liquid ratio, 1:30 w/v, temp 170°C, time 16 min, pH 7, Pressure 1 MPa         | Dialysis method    | SSP           | 24.57     | ND                     | 94        |
| *Eriobotrya Japonica*        | HWE; MAE; PLE; U-EAE; U-MAE | Solid-to-liquid ratio, 1:30 w/v, temp. 95°C, time 2 h; Solid-to-liquid ratio, 1:30 w/v, temp. 80°C, time 6.5 min, microwave power 500 W; Solid-to-liquid ratio, 1:10 w/v, temp. 55°C, time 40 min, pressure 1.8 MPa; Solid-to-liquid ratio, 1:40 w/v, time 20 min, ultrasound power 450 W; Cellulase dosage 50mg, time 20 min, ultrasound power 450 W; Time 6.5 min, ultrasound power 450 W, microwave power 500 W | ND; ND; ND; ND; ND; ND | LLP-W | 2.95      | 190-9971               | 95        |
|                              |                   |                                                                                       |                    | LLP-M         | 3.11      | 106-9799               |           |
|                              |                   |                                                                                       |                    | LLP-P         | 5.05      | 153-9178               |           |
|                              |                   |                                                                                       |                    | LLP-U         | 4.53      | 531.99                 |           |
|                              |                   |                                                                                       |                    | LLP-UE        | 4.73      | 1689,1366              |           |
|                              |                   |                                                                                       |                    | LLP-UM        | 4.93      | 3373,826               |           |
For polysaccharide extraction, water is applied to the extraction chamber with nitrogen purged by a purge valve, and the extraction is carried out at high pressure and high temperature (Fig. 5). After extraction, the extract was separated and cooled with a jacketed vessel attached to the extraction unit. High pressure and increased critical temperature improve the solubility and mass transfer rate, which results in a high extraction yield. Extraction time and solvent volume can be reduced with the help of PLE. Polysaccharides from Sagittaria sagittifolia L. leaves were extracted using PLE and showed a significant yield (Table 1) in the optimal conditions (a pH of 7, a temperature of 170 °C, a duration of 45 min, and a material-to-liquid ratio of 1:30). Pressurized-liquid extraction was applied to extract β-glucans from barley bran and showed a 16.39% extraction yield in the optimal conditions (pressure of 10 MPa, a temperature of 70 °C, and an extraction time of 9 min).
Supercritical-fluid extraction

Supercritical-fluid extraction (SFE) is an efficient extraction technique that gives an extraordinary yield and high purity and is commonly employed for the fractionation of low-molecular-weight polysaccharides. In SFE, gas-phase extraction, solvent (argon or carbon dioxide) is used, which is first cooled and then heated in a preheated column to maintain its high pressure and temperature, and then allowed to pass from the extraction chamber to extract polysaccharides from plant material (Fig. 6). Then, the extract is passed through the jacketed vessel where the extract is separated from plant material and cooled to room temperature. Supercritical fluid (SCF) is more applicable when carbon dioxide is used instead of argon, and polysaccharides are fractionated based on their solubility in carbon dioxide, and sometimes the solubility, and thus the yield, can be enhanced by introducing some organic solvents combined with water as a modifier. On a commercial scale, SFE is widely used for the selective fractionation of lactulose from different aldoses. Polysaccharides from Phyllostachys heterocycle leaves were extracted using SCF (supercritical CO₂) with ethanol as a modifier and there was a yield of 2.47% when the extraction was operated at a temperature of 50 °C and a pressure of 40 MPa for 2 h with 20 mL of ethanol. Supercritical-fluid extraction was applied for polysaccharide extraction from Artemisia sphaerocephala Krasch seeds and there was an 18.59% extraction yield when the extraction was performed under selected conditions such as a temperature of 45 °C, a pressure of 10 MPa, a CO₂ flow rate of 20 L/h, and an extraction time of 2 h. Polysaccharides from Ginkgo leaves were extracted using SFE-CO₂ and showed a 10.13% extraction yield when being processed with the optimized conditions including a duration of 99 min, a temperature of 63 °C, and a pressure of 42 MPa. For polysaccharide extraction, SFE is more advantageous than other extraction methods because it can provide maximum extraction efficiency with negligible degradation. Nonetheless, this extraction method is not very selective and the capital investment is high.

Enzyme-assisted extraction

Enzyme-assisted extraction (EAE) is another extraction method that is conventional, selective, specific, and efficient. Enzymes such as cellulase, amylase, hemicellulose, pectinase, and papain have the potential to break down the cell wall of plant material and release polysaccharides without affecting its structure by hydrolysis. In EAE, selected enzymes are applied to the extraction chamber under a controlled temperature to destroy the cell wall of plant material and hydrolyze the polysaccharides, which further passed through the centrifugation and filtration process in a jacketed vessel to make crude polysaccharides (Fig. 7). Enzyme-assisted extraction has several advantages over other traditional methods, such as high extraction yield, high reaction compatibility, short extraction time, low cost, greenness, and mild operation conditions. This extraction method has also attracted the attention of researchers because it is time and energy efficient and can be operated with a lower volume of extraction solvent. Sometimes, other constituents of plants, like proteins, lipids, phenolics, flavonoids, pigments, nucleic acids, and other small organic and inorganic compounds can interact with enzymes, which reduces the extraction
efficiency of polysaccharides. Moreover, temperature control is a critical parameter for achieving a high extraction yield, as treating polysaccharides at a high temperature can cause thermal degradation. Further studies are needed to overcome this problem. Lotus leaf-extract polysaccharides were extracted using EAE with three enzymes including amylase, cellulase, and pectinase, each 1% volume by weight of the leave powder, and showed extraction yields of 0.97, 1.17, and 1.11%, respectively, when the extraction was performed at a pH of 7 and a temperature of 50 °C for a duration of 48 h. Some other leaf-extracted polysaccharides using EAE are given in Table 1.

**Other methods**

**Combined extraction methods**

Combined extraction techniques can also be used for the extraction of polysaccharides to overcome the limitations...
of individual techniques. It is common to combine HWE with UAE, MAE, or EAE for polysaccharide extraction. Polysaccharides from *Eriobotrya japonica* leaves were extracted using ultrasound combined with enzyme-assisted or microwave-assisted extraction techniques (U-EAE and U-MAE). Using U-EAE with a cellulase enzyme dosage of 50 mg, an extraction time of 20 min, and an ultrasound power of 450 W, a yield of 4.73% was achieved, while U-MAE with an extraction time of 6.5 min, an ultrasound power of 450 W, and a microwave power of 500 W showed a 4.93% extraction yield.\(^9\) Polysaccharides from *Ginkgo biloba* leaves were extracted using ultrasound combined with enzyme-assisted (U-EAE) and showed a 12.85% extraction yield when being processed at a solid-to-liquid ratio of 1/50 (w/v), a temperature of 55 °C, an ultrasonic treatment time 30 min and a cellulase (0.8%) hydrolysis time of 40 min.\(^113\)

### Subcritical-water extraction

Subcritical-water extraction (SWE) is an extraction technique that is becoming popular due to its green nature. Another name for SWE is superheated-water extraction. Briefly, in SWE, polysaccharides can be extracted using a small quantity of solvent, especially water, under a high pressure ranging from 0.35 to 2.1 MPa and a high temperature ranging from 100 to 374 °C for a short time.\(^114\) Temperature, pressure, solid-to-liquid ratio, solvent flow rate, pH, and extraction time are important parameters that affect the extraction yield using SWE.\(^115\) Using SWE, polysaccharides from *Sagittaria sagittifolia* leaves were extracted, leading to a high yield of 24.57% at a solid-to-liquid ratio of 1:30 (w/v), a temperature of 170 °C, a pH of 7, and a pressure of 1 MPa, with an extraction time of 16 min.\(^9\) Subcritical-water extraction was successfully utilized for polysaccharide extraction from the stems of *Dendrobium* Lindl and showed a maximum extraction yield of 21.88% at a temperature of 129.83 °C, a pressure of 1.12 MPa, and a solid-to-liquid ratio of 1:25 (w/v), with an extraction time of 16.71 min.\(^116\) *Thlaspi arvense* leaves showed a 6.26% yield of selenium-containing polysaccharides when SWE was used at a temperature of 140 °C and a pressure of 8 MPa for 15 min.\(^117\)

### Ultra-high-pressure extraction

Ultra-high-pressure extraction (UPE) under high pressure at a low temperature can be used for the extraction of polysaccharides as a novel and green technique. It is one of the eco-friendly extraction methods approved by the US Food and Drug Administration (FDA) and has been widely adopted in the food industry.\(^118\) The UPE process can be operated at high pressure ranging from 100 to 1000 MPa with a low temperature of around 50 °C.\(^115\) This technique requires less time and solvent volume since high pressure enhances the mass transfer by disrupting the cell wall, and thus the polysaccharide extraction yield is increased. Ultra-high-pressure extraction showed a 3.07% polysaccharide yield from the root of *Morinda officinalis* at a pressure of 420 MPa and a solid-to-liquid ratio of 1:12 (w/v) for 6.5 min.\(^119\) In the optimal conditions using a UPE system, polysaccharides from *Litidis chinesis* Sonn. showed a 12.01% extraction yield when being processed at a solid-to-liquid ratio of 1:15 (w/v) and a pressure of 460 MPa for 17 min.\(^120\) The polysaccharide content in yellow tea leaf extracts increased by 1.31, 128.28, and 19.86% when UPE was operated at 200, 400, and 600 MPa respectively at 25 °C for 5 min.\(^121\)

### Ionic-liquid extraction

Paul Walden reported ionic liquids for the first time in 1914, and he is known as the father of ionic liquids (ILs).\(^122,123\) It was a time when scientists were not very familiar with the importance of ILs, but over time, the worth of these liquids expanded exponentially.\(^124\) In the present century, ILs are well known due to their versatile properties and wide range of applications in the field of chemical sciences.\(^122\) Ionic liquids are liquids formed by cations and anions linked with different types of chemical bonds and possesses a bundle of chemical and physical properties including low vapor pressure, good thermal stability, and high tunability, and can be used as solvents, catalysts, extraction liquids, etc., in various fields.\(^125\) They are composed of large organic cations and small organic or inorganic anions and have a melting temperature below 100 °C. The low melting temperature of ILs is suggested by their low ion symmetry composition and low charge density.\(^126\) Singh and Savoy\(^127\) classified ILs into several categories including task-specific ILs, chiral ILs, switchable polarity solvent ILs, bio-ILs, poly-ILs, energetic ILs, neutral ILs, protic ILs, metallic ILs, basic ILs, and supported ILs. Among other ILs, task-specific ILs including alkyl phosphate-type ILs and imidazolium-based hydrophilic ILs have been most used in the IL extraction (ILE) of polysaccharides.\(^127\) Ionic-liquid extraction has been used frequently for cellulose extraction from biomass using those hydrophilic ILs that have good hydrogen-bond-acceptor capability and moderate hydrogen-bond-donor behavior because the solubility of cellulose is increased in those ILs at room temperature.\(^128\) The extraction yields of cellulose from corn stover using three ILs, tetra-butylphosphonium 2-ethylhexanoate ([P\(_{1444}^+\)]{[EH]}), dodecyl-3-methylimidazolium bis(2,4,4-tri-methyl-pentyl)phosphinate ([C\(_4\)mim][P2,3(PO3)]), and 1-decyl-3-methylimidazolium bis(2,4,4-tri-methyl-pentyl)phosphinate ([C\(_{10}\)mim][P2,3(PO3)]) were 84, 61.1, and 44%, respectively when the extraction was...
Carried out at a temperature of 80 °C for a duration of 2 h.\textsuperscript{129} Cellulose was extracted from Zoysia japonica using allyl-3-methylimidazolium chloride and showed a 71% extraction yield when being immersed at a temperature of 80 °C for 2 h.\textsuperscript{130} Polysaccharides from Japanese cedar extracted using ILE with 1-(3-methoxypropyl)-3 methylimidazolium ethyl ethylphosphonate showed a 15% extraction yield when being processed at a temperature of 100 °C and an IL dosage of 2 g under a nitrogen environment for 24 h.\textsuperscript{131} Wheat bran polysaccharides were extracted using ILE and showed a 16.1% extraction yield when being immersed in 1,3-dimethylimidazolium methyl methylphosphonate ([Cimim][(MeO)(Me)PO\textsubscript{3}]) at a temperature of 80 °C for 2 h.\textsuperscript{132} Polysaccharides from Mentha haplocalyx were extracted using three different solutions such as citric acid (pH 3) at 95 °C for 3 h, 5% NaOH/0.05% NaBH\textsubscript{4} at 25 °C for 3 h, and 0.9% NaCl at 95 °C for 3 h, and the yields were 7.28, 9.37, and 7.78% respectively.\textsuperscript{133} Plotka-Wasylka et al.\textsuperscript{133} reported that some of the ILs were considered to be green, non-flammable, non-volatile, and stable in air and water, but recently, many of them have been found to be flammable, volatile, unstable, and even toxic. On a large scale, ILE cannot be used because some ILs are hazardous and non-biodegradable, and the large-scale synthesis of ILs is expensive and difficult.\textsuperscript{123}

**Deep eutectic solvents**

Deep eutectic solvents (DESs) are alternatives to ILs and are known to be less hazardous, less expensive, more stable, and biodegradable in comparison with ILs.\textsuperscript{134} Deep eutectic solvents possess some advantages like the ease of synthesis, non-flammability, non-volatility, low cost for large-scale production, high biocompatibility, and the wide availability of their primary ingredients.\textsuperscript{135} They represent a new class of IL formed from a eutectic mixture of Lewis or Bronsted acids and bases, with several anionic and/or cationic species, and they differ from ILs, which are composed of one type of discrete anion and cation.\textsuperscript{136} According to the first described concept, DESs are liquids formed by mixing a variety of quaternary ammonium salts and carboxylic acid.\textsuperscript{137} They are synthesized by combining hydrogen-bond donors (HBDs) and hydrogen-bond acceptors (HBAs) to form eutectic mixtures.\textsuperscript{138} Based on the complexing agent, DESs are classified into four categories including Type I (composed of a quaternary ammonium salt (QAS) and a metal chloride), Type II (composed of a QAS and a metal chloride hydrate), Type III (composed of QAS and a hydrogen bond donor), and Type IV (composed of a metal chloride and a hydrogen bond donor).\textsuperscript{138} The most widely used hydrogen-bond acceptor in DESs is choline chloride (ChCl), and choline-derived DESs have been reported extensively for the dissolution of cellulose.\textsuperscript{139} Hemicellulose and amorphous cellulose were extracted from rice straw and showed 16.71 and 9.60% extraction yields when being treated with ChCl/urea at a temperature of 130 °C for 4 h.\textsuperscript{140} Polysaccharides from Fucus vesiculosus were extracted with a DES of ChCl and 1,4-butanediol at a molar ratio of 1:5, and showed an 11.63% extraction yield when being processed at a temperature of 168 °C for 35 min.\textsuperscript{141} Lotus leaf polysaccharides showed a 5.38% extraction yield when being extracted with a DES of ChCl and ethylene glycol at a molar ratio of 1:3, a solid-to-liquid ratio of 1:31 (w/v), and a temperature of 92 °C for 126 min.\textsuperscript{142} In DES extraction, two or more organic or inorganic liquids or combinations of both types of liquid can also be used to obtain specific properties for polysaccharide extraction.\textsuperscript{143}

**Pulsed electric field-assisted extraction**

In pulsed electric field-assisted extraction (PEF), an electric field ranging from 0.1 to 80 kV is applied to plant cells. This permeabilizes the cell membrane to release constituent compounds such as carbohydrates, polyphenolic compounds, and flavonoids in the solvent system at ambient temperature.\textsuperscript{115} Pulsed electric field-assisted extraction is a novel, nonthermal and efficient extraction method that is capable to extract high-purity polysaccharides within seconds with less energy.\textsuperscript{144} Corn bran polysaccharides were extracted using water-assisted and enzyme-assisted PEF, which showed 6.4 and 15.36% extraction rates in the optimal conditions (a solid-to-liquid ratio of 1:42 (w/v), an electric field intensity of 25 kV/cm, and an electric field frequency of 1080 Hz).\textsuperscript{145} Corn silk polysaccharides were extracted using PEF and a 7.31% extraction yield was achieved when the extraction was carried out at an electric intensity of 30 kV/cm and a solid-to-liquid ratio of 1:50 (w/v) for 6 μs.\textsuperscript{146}

**Negative pressure cavitation**

In this extraction method, cavitation is performed by applying negative pressure as a part of hydrodynamic cavitation. Negative pressure cavitation (NPC) involves the developed cavitation to increase the mass transfer of bioactive compounds including polysaccharides from plant material to the extraction solvent by colliding the surface of plant material.\textsuperscript{147} Temperature, pressure, time, solvent concentration, and solid-to-liquid ratio are parameters that affect the extraction yield of polysaccharides. Polysaccharides from Astragalus membranaceus roots were extracted using NPC and showed a 16.74% extraction yield when the extraction was performed under parameters such as a pressure of −0.068 MPa, a temperature of 64.8 °C, a solid-to-liquid
ratio of 1:13.4 (w/v), a homogenization time of 70 s, and an extraction time of 53 min.\textsuperscript{148}

**Comparison of different extraction methods**

Carbohydrates are produced in plants by photosynthetic CO\textsubscript{2} fixation and are a central source of energy in the global bioeconomy. There are three reasons for the importance of investigating polysaccharides. First, the emergence of the bioeconomy highlights the contribution of natural products, especially biobased products. Second, while polysaccharides have been utilized widely in material science, health care, food, and nutrition, it is important to evaluate the exceptional properties of polysaccharides to open possible routes to novel applications. One more reason is associated with environmental concerns, and regarding this, the adoption of polysaccharides can contribute to sustainability because of their ubiquitous presence and renewability.\textsuperscript{149} The several potential benefits include an increase in biodiversity, food safety, sustainability, and fuel production, and a decrease in CO\textsubscript{2} emission and pollution, which could be achieved by proper utilization of polysaccharides. Polysaccharides are widely distributed due to their different structures and are classified based on origin, shape, structure, charge, monosaccharide unit, and chemical and functional properties.\textsuperscript{5} Generally, polysaccharides obtained from plant leaves with long chains and complex structures with branches have attracted much attention because they have the potential to regulate a variety of biochemical functions like cell proliferation, immune response, and cell differentiation, inflammation, and adhesion.\textsuperscript{150} Biochemical functions including cell proliferation, immune response, cell differentiation, inflammation, and adhesion are related to the biological activities of polysaccharides. Polysaccharides with long chains, complex structures, and clusters of branches have been reported to perform excellent biological activities in comparison with short-chain, linear, and simple structured polysaccharides.\textsuperscript{18,151} The recovery of such polysaccharides without disturbing their composition and structure is much more important. In this sense, a bundle of extraction methods discussed above can be used for polysaccharide extraction from plant leaves, but this section differentiates the extraction methods according to their performance. Hot-water extraction is an easy and commonly used extraction method but it requires a high temperature and time, whereas these issues were not seen with UAE and MAE. Mulberry leaf polysaccharides were extracted using HWE and MAE, and HWE has an 18.64% extraction yield, whereas MAE provides a yield of 9.41% under the optimal conditions (Table 1).

**Table 1.** Comparison of different extraction methods for polysaccharides. (continued)

Hot-water extraction is superior to MAE considering extraction yield. High-molecular-weight polysaccharides could be extracted using HWE and EAE, whereas UAE and MAE mostly tend to extract low-molecular-weight polysaccharides (Table 1). Hot-water extraction and EAE were utilized respectively to extract polysaccharides from Camellia sinensis. A 1.28% extraction yield was obtained using HWE while an extraction yield of 4.08% using EAE under the optimal conditions. Enzyme-assisted extraction performed better over HWE regarding extraction yield and molecular weight distribution (Table 1). Corn silk polysaccharides were extracted using HWE and PEF respectively and showed a 5.46% yield at 100 °C temperature with an extraction duration of 60 min using HWE, while a 7.31% yield was obtained using PEF under the optimal conditions such as an electric field intensity of 30 kV/cm and a reaction time of 6 μs.\textsuperscript{146} Pulsed electric field-assisted extraction is a fast, nonthermal and efficient extraction technique but it has not been widely utilized for polysaccharide extraction. It is currently not feasible on a large scale due to the complexity of setting it up.

Polysaccharides from Eriobotrya japonica leaves have been extracted using HWE, MAE, PLE, UAE, U-EAE, and U-MAE, respectively (Table 1). Extraction yields of 2.95, 3.11, 5.05, 4.53, 4.73, and 4.93% were obtained using HWE, MAE, PLE, UAE, U-EAE, and U-MAE, under the following operating conditions: solid-to-liquid ratio 1:20 (w/v), temperature 95 °C, and extraction time 2 h for HWE; solid-to-liquid ratio 1:30, temperature 80 °C, time 6.5 min, and microwave power 500 W for MAE; solid-to-liquid ratio 1:10, temperature 55 °C, time 40 min, and pressure 1.8 MPa for PLE; solid-to-liquid ratio 1:40, time 20 min, and ultrasound power 450 W for UAE; cellulase dosage 50 mg, time 20, and ultrasound power 450 W for U-EAE; and time 6.5 min, ultrasound power 450 W, and microwave power 500 W for U-MAE. The results showed that PLE provided the highest extraction yield in the optimal conditions. Ginkgo biloba leaf polysaccharides were extracted using three extraction methods including UAE, EAE, and U-EAE and showed different yields of 8.36, 7.92, and 12.85% respectively at the optimal conditions of a solid-to-liquid ratio of 1/50 (w/v), a temperature of 50 °C, ultrasound treatment for 30 min, and enzyme hydrolysis with 0.8% cellulase for 40 min.\textsuperscript{113} The extraction yield of polysaccharides has increased significantly with the combination of two methods, which suggests that combining extraction methods provides other possible options for the extraction of polysaccharides on a large scale. In comparison, HWE, MAE, and PLE extracted high-molecular-weight polysaccharides while UAE, U-EAE, and U-MAE extracted low-molecular-weight polysaccharides.
Pressurized-liquid extraction and EAE might therefore be the most acceptable extraction techniques for the extraction of polysaccharides from plant leaves on a commercial scale.

**Purification of polysaccharides**

After extraction followed by the ethanol precipitation process, purification and fractionation are important procedures to remove residues of other constituents such as proteins, lipids, phenolics, flavonoids, pigments, nucleic acids, and other small organic and inorganic compounds conjugated with plant polysaccharides. Conventional methods used for the purification of crude polysaccharides include ethanol treatment, hydrogen peroxide treatment, activated carbon treatment, amylase hydrolysis, ultrafiltration through a membrane, dialysis against water (ultrapure, distilled), and Sevag reagent treatment ((n-butanol and chloroform, 1:5). Polysaccharides can be fractionated based on their size, charge, and chemical interaction, with the help of column chromatography, gel permeation chromatography, ion-exchange chromatography, and affinity chromatography.

**Column chromatography**

Column chromatography is one of the simplest, conventional methods used for the fractionation of polysaccharides, and nowadays it has attracted wide attention due to its excellent performance in the fractionation of plant polysaccharides. Cellulose is a commonly used stationary phase in column chromatography. It is equilibrated with ethanol to avoid the nonspecific adsorptive forces of cellulose molecules to polysaccharides. Fractions of polysaccharides can be eluted by using state-of-the-art eluents such as water, buffers, and some organic solvents. Polysaccharides, with short chains and low molecular weight, have weak interactions with ethanol-equilibrated cellulose and are eluted first. Polysaccharides have moderate branches and possess medium molecular weight. They interact moderately with ethanol-equilibrated cellulose and are eluted in the second place. Polysaccharides have long branches and possess high molecular weight. They have strong interactions with ethanol-equilibrated cellulose and are eluted last. However, the lower the cellulose particle size, the greater is the surface area, and the higher is the number of theoretical plates. Hence there is higher fractionation efficiency for polysaccharides. The low flow rate and time-consuming behavior of column chromatography make it less usable.

**Gel permeation chromatography**

Polysaccharides can be fractionated based on their size and shape by using gel permeation chromatography (GPC). In GPC, gels with pores of different sizes are used to separate polysaccharide molecules. Polysaccharides with large molecules and high molecular weight do not enter the pores of gel and are eluted first. Polysaccharides with small molecules and low molecular weight obstruct the pores of the gel and are eluted last. Some commonly used gel-packing materials include Sepharose, Sephacyr, Superdex, Bio Gel, and Sephadex. The selection of gel is strongly dependent on the nature and source of polysaccharides that are to be fractionated. For polysaccharide fractionation, GPC with a refractive index detector (RID) is most widely used. The peaks of different polysaccharide fractions are generated based on RID elution by using deionized/distilled water and sodium chloride solution with different concentrations along with buffer solutions. In GPC-RID, some ionic solvents can be run before the introduction of samples to reduce the nonspecific adsorptive forces of the gel to polysaccharides, and hence increase the purity. There is no doubt that GPC is a powerful analytical technique for polysaccharide fractionation but it possesses some limitations such as expensive instrumentation, low efficiency, a lack of automation, and difficulty in scaling up.

**Ion-exchange chromatography**

Polysaccharides can be fractionated with the help of ion-exchange chromatography (IEC). In IEC, polysaccharides are separated based on charge and polarity index. Ion-exchange chromatography is operated with either cation exchange resins or anion exchange resins but anion exchange resins are commonly preferred for polysaccharides fractionation. Anion resins separate neutral and acidic fractions of polysaccharides. Acidic polysaccharides having a high uronic acid content can bind strongly to anion resin, and neutral polysaccharides do not have interaction with anion resin and hence are eluted first. Furthermore, neutral and acidic polysaccharides are fractionated by using gradient elution practices with a combination of different ionic eluents. Diethylaminoethyl (DEAE) containing anion exchange resins such as DEAE-cellulose, DEAE-Sepharose, DEAE-Sepharose fast flow, DEAE-Sephadex, DEAE-Sephadex fast flow, and Q-Sepharose are most widely used for polysaccharide fractionation. The selection of an anion exchanger depends on small-scale experimental trials and the concentration of uronic acid in polysaccharides.

**Affinity chromatography**

Selective liquid adsorption chromatography, commonly known as affinity chromatography (AC) is another analytical tool for polysaccharide fractionation. In AC, the
polysaccharides are separated based on adsorptive forces to the stationary phase.\textsuperscript{165} Immobilized ligands act as stationary phases in affinity chromatography. Many commercially available immobilized lectins can be used as ligands in AC. Practically, Concanavalin A, wheat germ agglutinin, and Sepharose have been used for glycoprotein fractionation.\textsuperscript{164} Many standard polysaccharides such as mannan, chitin, alginate, and other polysaccharides from \textit{Azospirillum brasilense} and \textit{P. aeruginosa} interact well with these ligands for fractionation.\textsuperscript{165,166} Affinity chromatography can be used as a potential separating tool on small and large scales but the selection of ligands for typical polysaccharides might be time-consuming. This fractionation tool demands more research work in the future to explore further fundamental trends of polysaccharides fractionation.

**Antioxidant values of polysaccharides**

A large number of free radicals produced in different products during chemical degradation, which occurs under different conditions, are detrimental to the entire product and the human body.\textsuperscript{166} These free radicals are harmful to the entire body and generate oxidative stress in biological systems.\textsuperscript{166,167} Free radicals can oxidize biological macromolecules like lipids, proteins, and carbohydrates, causing serious biological disorders such as inflammation, aging, cancer, hepatotoxicity, and diabetes.\textsuperscript{168} Polysaccharides play a meaningful role as modifiers towards biological response and reduce or inhibit oxidative stress by scavenging free radicals.\textsuperscript{18} Polysaccharide antioxidants can be used to prevent a wide range of oxidative disorders as well as preservatives in cosmetic and food products.\textsuperscript{169} Previous studies indicate that polysaccharides extracted from plant leaves have good antioxidant activities by scavenging free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, 2,2-azino-\textit{bis}(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and superoxide, and have also shown excellent reducing power (Table 2).

1,1-Diphenyl-2-picrylhydrazyl radical antioxidant activity

The antioxidant assay based on DPPH is a direct, simple, and reliable method. DPPH is a very stable free radical because free electrons are delocalized over the whole molecule without dimerizing, and in the presence of hydrogen species, the DPPH radical is reduced into 1,1-diphenyl-2-picrylhydrazine by changing the color from violet to yellow.\textsuperscript{170,171}

Plant leaf-extract polysaccharides have good DPPH antioxidant activity at the optimal dose (see Table 2). At a 2.8 mg/mL concentration, the maximum DPPH antioxidant activity of 94% was obtained for Zagros oak leaf-extracted polysaccharides.\textsuperscript{86} Besides, polysaccharides extracted from leaves of \textit{Leonurus cardiaca} showed 92.8% DPPH antioxidant activity when it was applied with a concentration of 12.14 mg/mL.\textsuperscript{46} In the same way, polysaccharides separated from leaves of \textit{Mentha haplocalyx}, \textit{Acanthopanax senticosus}, and mulberry showed DPPH antioxidant activities of 91.88, 91.75, and 91% when they were used at concentrations of 2, 2, and 0.24 mg/mL, respectively.\textsuperscript{39,47,82}

**Hydroxyl radical antioxidant activity**

The hydroxyl radical (OH\textsuperscript{•}) is one of the most reactive radicals that can attack the biological system.\textsuperscript{172} Hydroxyl radical scavenging assay is based on the inhibition of OH\textsuperscript{•} radicals that can be produced during the Fenton reaction. In this reaction, hydrogen peroxide is introduced to ferrous ions, which results in ferric ions through the oxidation process. This oxidation is based on the availability of hydroxyl radicals to oxidize Fe\textsuperscript{2+} to Fe\textsuperscript{3+}. In other words, the production of Fe\textsuperscript{3+} ions increased by increasing the number of hydroxyl radicals.\textsuperscript{173} Hydroxyl radicals can be captured with the help of antioxidant species that donate hydrogen atoms and this capturing can be determined by the salicylic acid method, which forms a purple-color complex (absorb at 510 nm) with Fe\textsuperscript{3+} ions. If OH\textsuperscript{•} radicals are scavenged by species under study, the absorbance at 510 nm is reduced compared to the control solution.

The hydroxyl radical antioxidant activity of \textit{Lilium lancifolium} leaf-extract polysaccharides was found to be about 96.16% when it was loaded with a concentration of 3 mg/mL.\textsuperscript{41} Leaf-extract polysaccharides of \textit{Leonurus cardiaca} at a 13.5 mg/mL concentration showed the best OH\textsuperscript{•} scavenging activity (about 94.8%), and \textit{Althaea officinalis} leaf-extract polysaccharides at a concentration of 20 mg/mL showed 94.8% OH\textsuperscript{•} scavenging activity.\textsuperscript{46,67} Polysaccharides isolated from epimedium leaves with different fractions were found to show the maximum OH\textsuperscript{•} radical antioxidant activity (94.60%) at a concentration of 8 mg/mL.\textsuperscript{81} Furthermore, leaf-extract polysaccharides of \textit{Ilex latifolia}, \textit{Plantago ovata}, and \textit{Cyclocarya paliurus} had 92.13, 91.7, and 90.16% hydroxyl radical antioxidant activities with their concentrations being 4, 0.1, and 0.24 mg/mL, respectively.\textsuperscript{137,55}

2,2-Azino-\textit{bis}(3-ethylbenzothiazoline-6-sulfonic acid) radical antioxidant activity

By oxidizing ABTS with potassium persulfate, a nitrogen-centered radical cation of ABTS is produced. When the electron-donating species like polysaccharides come...
Table 2. Antioxidant activity of plant leaf-extract polysaccharides.

| Leaves           | Compound name | DPPH* (%) at the optimal dose | OH* (%) at the optimal dose | ABTS* (%) at the optimal dose | O−2• (%) at the optimal dose | FRAP (absorbance at 700 nm) | Reference |
|------------------|---------------|-------------------------------|----------------------------|-----------------------------|-----------------------------|---------------------------|-----------|
| Ginkgo biloba    | GBPS-2        | 38.35                         | 34.43                      | 74.34                       | 60.12                       | ND                        | 34        |
|                  | GBPS-3        | 44.59                         | 36.62                      | 82.01                       | 64.34                       | ND                        |           |
| Olive            | OLP           | 80                            | ND                         | ND                          | ND                          | 3.00                      | 36        |
| Plantago ovata   | W             | 22.7                          | 41.3                       | 47.2                        | ND                          | ND                        | 37        |
|                  | A             | ND                            | 38.2                       | 55.5                        | ND                          | ND                        |           |
|                  | W1            | ND                            | 80.9                       | 51.8                        | ND                          | ND                        |           |
|                  | A1            | 15.2                          | 76.5                       | 54.6                        | ND                          | ND                        |           |
|                  | W2            | 38.2                          | 91.7                       | 58.3                        | ND                          | ND                        |           |
|                  | A2            | 25.7                          | 86.4                       | 51.7                        | ND                          | ND                        |           |
| Maca             | MPL-1         | 62.1                          | 82.71                      | ND                          | 76.67                       | ND                        | 38        |
|                  | MPL-2         | 59.67                         | 79.07                      | ND                          | 52.62                       | ND                        |           |
| Lilium lancifolium| LLP-1        | 55.71                         | 86.46                      | ND                          | 93.26                       | ND                        | 41        |
|                  | LLP-2         | 70.13                         | 95.85                      | ND                          | 91.49                       | ND                        |           |
|                  | LLP-3         | 78.15                         | 96.16                      | ND                          | 96.83                       | ND                        |           |
| Lepidium meyenii | LMLP          | 68.42                         | ND                         | ND                          | ND                          | 0.079                     | 40        |
| Morus alba       | MLP           | 68.21                         | 88.85                      | 99.33                       | 84.47                       | ND                        | 43        |
|                  | MLP-3a        | 44.96                         | 57.89                      | 79.81                       | 71.91                       | ND                        |           |
|                  | MLP-3b        | 60.17                         | 68.12                      | 90.47                       | 75.25                       | ND                        |           |
| Paris polyphylla | PPLP          | 84.73                         | 79.04                      | ND                          | 76.09                       | ND                        | 44        |
| Cyclocarya paliurus| CP           | 87.21                         | ND                         | ND                          | ND                          | ND                        | 45        |
|                  | AC-Cp-1       | 93.55                         | ND                         | ND                          | ND                          | ND                        |           |
|                  | AC-Cp-2       | 89.69                         | ND                         | ND                          | ND                          | ND                        |           |
|                  | AC-Cp-3       | 90.27                         | ND                         | ND                          | ND                          | ND                        |           |
| Leonurus cardiaca| LCLP          | 92.8                          | 94.8                       | ND                          | ND                          | ND                        | 46        |
| Mulberry         | MLCP          | 91                            | 86                         | ND                          | ND                          | ND                        | 47        |
| Cyclocarya paliurus| CP           | ND                            | 70.13                      | ND                          | 51.21                       | ND                        | 51        |
|                  | CM-Cp-1       | ND                            | 73.23                      | ND                          | 47.26                       | ND                        |           |
|                  | CM-Cp-2       | ND                            | 61.97                      | ND                          | 42.51                       | ND                        |           |
|                  | CM-Cp-3       | ND                            | 60.92                      | ND                          | 40.73                       | ND                        |           |
| Ilex latifolia   | ILPs-1        | 75.14                         | 92.13                      | ND                          | 78.92                       | ND                        | 55        |
|                  | ILPs-2        | 24.70                         | 57.15                      | ND                          | 35.9                        | ND                        |           |
|                  | ILPs-3        | 41.30                         | 57.95                      | ND                          | 57.13                       | ND                        |           |
|                  | ILPs-4        | 52.15                         | 70.32                      | ND                          | 65.17                       | ND                        |           |
|                  | ILPs-5        | 63.72                         | 82.19                      | ND                          | 70.47                       | ND                        |           |
| Bruguiera gymnorrhiza| BGP         | ND                            | 63.3                       | 62.2                        | 62.4                        | ND                        | 57        |
| Phyllostachys pubescens| NPs       | 85.90                         | ND                         | 99.98                       | ND                          | 0.550                     | 75        |
|                  | Aps           | 69.38                         | ND                         | 35.73                       | ND                          | 0.047                     |           |
|                  | MPs           | 64.53                         | ND                         | 99.99                       | ND                          | 0.251                     |           |
| Guava            | GLP           | 56.38                         | ND                         | 51.73                       | ND                          | ND                        | 76        |
| Suaeda fruticosa | SFP           | 69.5                          | ND                         | 69                           | ND                          | ND                        | 77        |
| Hibiscus         | HRLP          | 64.73                         | 65.32                      | ND                          | ND                          | ND                        | 80        |
| Epimedium        | EP1           | 63.95                         | 82.71                      | ND                          | 31.52                       | ND                        | 81        |
|                  | EP2           | 79.94                         | 94.60                      | ND                          | 49.50                       | ND                        |           |
|                  | EP3           | 83.16                         | 88.14                      | ND                          | 68.48                       | ND                        |           |

(Continued)
close to this radical, this radical accepts an electron from polysaccharides and transforms into a non-radical form. The extent of ABTS radical scavenging is assayed by the reduction of the blue-green solution of ABTS at 734 nm using a spectrophotometer.173

The ABTS radical antioxidant activity of *Phyllostachys pubescens* leaf-extract polysaccharides at a concentration of 3 mg/mL was found to be 99.98%.75 *Morus alba* leaf-extract polysaccharide showed high ABTS-radical-scavenging activity of 99.33% when being applied at a concentration of 4 mg/mL.43 Polysaccharides separated from leaves of *Silphium perfoliatum* displayed 93.69% ABTS radical antioxidant activity at a low concentration of 1.2 mg/mL.80 Likewise, polysaccharides extracted from leaves of *Ginkgo biloba, Bruguiera gymnorrhiza,* and guava exhibited ABTS radical antioxidant activities of 82.01, 62.2, and 51.73% when they were applied at concentrations of 4, 5, and 0.1 mg/mL, respectively.34,57,76

### Superoxide radical antioxidant activity

Superoxide radicals (O$_2^-$) belong to one of the most toxic radicals commonly generated during biological and photochemical reactions.84 The superoxide radical scavenging potential of polysaccharides is commonly assayed by the NADH–NBT–PMS system. In this system, the superoxide radical is generated by the reaction of β-nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS). The PMS is reduced by NADH and generates O$_2^-$, which is further reduced by nitroblue tetrazolium (NBT).174,175 This radical is scavenged by antioxidant species like polysaccharides and decreases the reducing extent of NBT, which is monitored at 569 nm using a spectrophotometer.82

The superoxide radical antioxidant activity of *Lilium lancifolium* leaf-extract polysaccharides was found to be 96.83% when it was applied at a concentration of 1 mg/
ions are reduced into

In the same way, the

With the presence

−

187

181

176.

ions, which can be monitored by the ferric-ferrocyanide

179

180

185

Fe

2+

Pectin is extensively used in child food items like
to Fe

3+. At low concentrations, pectin binds water to

186

43

182, 183

It

Moso bamboo leaf-extract polysaccharides showed superoxide radical antioxidant activity of 86.13% at a concentration of 1 mg/mL.60 Leaf-extract polysaccharides of Ilex lattifolia, Bruguiera gymnorrhiza, epimedium, and Rhododendron aganniphum showed 78.92, 62.4, 68.48, and 84.87% superoxide radical antioxidant activity when they are utilized at concentrations of 4, 5, 6.5, and 0.2 mg/mL, respectively.55,57,81,84

**Ferric reducing antioxidant power**

Ferric reducing antioxidant power (FRAP) is an inexpensive, simple, and sensitive single electron transfer-based assay.176 It is based on the reduction of Fe

3+ to Fe

2+. With the presence of species like polysaccharides, Fe

3+ ions are reduced into Fe

2+ ions, which can be monitored by the ferric-ferrocyanide (KFe[Fe(CN)]

6) spectrophotometric method at 700 nm. The greater the reducing potential of polysaccharides, the greater is the absorbance of the ferric-ferrocyanide complex at 700 nm.

Ferric reducing antioxidant power of plant leaf-extract polysaccharides can be described in terms of absorbance at 700 nm. The greater the absorbance, the greater is the reducing power of plant leaf-extract polysaccharides (Table 2). Polysaccharides obtained from olive leaves showed a high reducing potential (3 absorbances at 700 nm) at a low concentration of 0.7 mg/mL.36 In the same way, the reducing potential of plant leaf-extract polysaccharides extracted from different sources follows the order of Gynura procumbens > Silphium perfoliatum > Mentha haplocalyx > Malva sylvestris > Acanthopanax senticosus > Phyllostachys pubescens at the optimal concentration.

**Large-scale applications of plant polysaccharides**

Starch, hemicellulose, cellulose, pectin, and gums are the most important plant polysaccharides for industrial applications. Starch is a fundamental polysaccharide for human life and is found in leaves, root tubers, fruits, and seeds of plants as a storage polysaccharide. Starch is composed of amylose and amylopectin, which are glucose chains with different lengths and degrees of branching depending on origin.178 In the food industry, starch is widely used as an additive to improve the thickening and adhesion of liquid and paste products. Cationic starches as wet-end additives are used extensively in the paper industry. In the textile industry, starch is used for wrap sizing and fabric printing. Starches are used widely as excipients, diluents, disintegrants, binders, lubricants, glidants, and drug deliverers in pharmaceutical products.179

Hemicellulose polysaccharides (xylglucans) have a linear structure and are present in the cell walls of higher plants.180 Tamarind seed xylglucan is one of the most studied hemicellulose polysaccharides regarding rheological behavior and different applications.181 Xylglucans are widely used in the food industry (as, e.g., stabilizers, thickeners, gelling agents, and modifiers), the pharmaceutical industry (for drug delivery systems due to hydrophilic and mucoadhesive properties), and the cosmetic industry (as ultraviolet protective agents).181

One of the richest polysaccharides in nature is cellulose, which is typically found in plant and fungi cell walls and is also synthesized by some bacteria.182,183 In cellulose, glucose molecules are joined together by β(1→4) linkage. It is hydrophilic, biodegradable, and insoluble in water and most organic solvents.184 Cellulose acetates, cellulose nitrates, cellulose propionates, cellulose sulfates, and cellulose ethers are the most commonly used derivatives of cellulose.185

The most significant types of cellulose that are widely used for commercial purposes are cellulose esters and cellulose ethers.185 Cellulose is used on a large scale in the paper industry (to make paper), the textile industry (e.g. for making fabric), the pharmaceutical industry (e.g. as an additive, thickening agent, and drug delivery and gelling agent), and the food industry (e.g. as an additive, thickening agent, and viscosifier). The potential application of pectins – structural acidic heteropolysaccharides contained in the primary and middle lamella and cell walls of terrestrial plants – are being recognized increasingly and have been widely studied due to their complex structures.185 Commercially, pectin can be extracted from citrus peels and some fruits, like apple under acidic conditions.100 Pectin is widely used in the food industry as a gelling agent, stabilizer, thickening agent, and viscosifier.186 At low concentrations, pectin binds water to form gel.187 Pectin is extensively used in child food items like toffees, jellies, and jams.

Gums are high-molecular-weight macromolecules obtained from plant exudates, which are soluble in water and have stabilizing and thickening effects.188 In gums, monomers like glucose, mannose, galactose, xylose, amylose, and arabinose are joined together by glycosidic linkage with a perspective anomeric conformation. Gums differ in their properties (e.g. pH, solubility, gelling power, and viscosity) and source. Some gums are found in associated forms with terpenoids or proteins.189 Gum Arabic, gum tragacanth, gum karaya, and
gum ghatti are obtained from plant exudates whereas locust bean gum, guar gum, and tamarind are obtained from the seeds of plants. Gums are widely used in the food industry, pharmaceutical industry, cosmetic industry, and the chemical industry.\cite{190}

**Conclusions and future projections**

This review highlighted the importance of plant leaf-extract polysaccharides and explored major aspects of the utilization of polysaccharides, including extraction, purification, and their antioxidant potential. Several commonly used extraction methods such as HWE, MAE, UAE, PLE, SFE, and EAE provide meaningful extraction efficiency for plant leaf-extract polysaccharides. All these extraction techniques have appropriate uses but consideration of several factors differentiates them. Hot-water extraction is time-consuming and requires high temperatures to obtain the best polysaccharide extraction efficiency, whereas UAE and MAE are time-saving. Normally, UAE and MAE favor extracting low-molecular-weight polysaccharides, while HWE is better at extracting high-molecular-weight polysaccharides. In our opinion, EAE and PLE are the only extraction methods that can be used for polysaccharide extraction on a large scale. Pressurized-liquid extraction and SWE are very similar to each other in terms of basic principles. Subcritical-water extraction is operated at high temperature and so it is also called superheated extraction. At high temperatures, extraction efficiency is reduced due to the degradation of polysaccharides. Although ILE provides good extraction efficiency for selective polysaccharides like cellulose, chitin, and pectin, technical development is still needed to make it fully cost effective. Supercritical-fluid extraction, UAE, and NPC are complicated processes and are not being widely applied to extract plant leaf polysaccharides due to high costs and some operational limitations. Pulsed electric field-assisted extraction is a novel, nonthermal, efficient and fast extraction method that is capable of extracting high-purity polysaccharides within seconds by consuming less energy, but further research is needed to use it on a commercial scale. Numerous conventional techniques can be used to remove residues of other constituents like proteins, lipids, phenolics, flavonoids, pigments, nucleic acids, and other small organic and inorganic compounds that are conjugated with leaf polysaccharides, but there are still some gaps in these methods related to their optimization. The major impurities in leaf polysaccharides are proteins. Hence, several quick, feasible, and novel protein-removal protocols should be established. The high antioxidant activity of plant leaf-extract polysaccharides suggests that garden waste can be regarded as a healthy source of antioxidant polysaccharides. Finally, this review advises developing a commercial-scale setup to convert garden waste into polysaccharides that can play a vital role in functional applications as value-added products and stabilizing agents.

**Acknowledgement**

This work was supported by the Engineering and Physical Sciences Research Council (EPSRC) (grant number EP/V002236/2).

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