International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Review

Isolation, structural characterization and bioactivities of naturally occurring polysaccharide–polyphenolic conjugates from medicinal plants—A reivew

Jun Liu*, Ruyu Bai, Yunpeng Liu, Xin Zhang, Juan Kan, Changhai Jin*

College of Food Science and Engineering, Yangzhou University, Yangzhou 225127, Jiangsu, China

ARTICLE INFO

Article history:
Received 5 July 2017
Received in revised form 2 September 2017
Accepted 15 October 2017
Available online xxx

Keywords:
Biological activity
Conjugate
Polyphenolic
Polysaccharide
Structural characterization

ABSTRACT

In recent years, several medicinal plants have been demonstrated as valuable resources of naturally occurring polysaccharide–polyphenolic conjugates. For the first time, this article introduces recent advances of polysaccharide–polyphenolic conjugates isolated from different medicinal plants. The isolation, purification, structural characterization and biological activities of polysaccharide–polyphenolic conjugates are introduced in details. In general, polysaccharide–polyphenolic conjugates can be isolated by hot water or alkaline extraction followed by purification through anion exchange chromatography or gel filtration chromatography. The structures of conjugates are usually characterized by chemical composition analysis, UV–vis, Fourier-transform infrared and nuclear magnetic resonance spectroscopy. Moreover, polysaccharide–polyphenolic conjugates exhibit several biological activities including anticoagulant, antioxidant, radioprotective, anti-platelet, antitussive and bronchodilatory effects. Therefore, polysaccharide–polyphenolic conjugates isolated from medicinal plants are certain to have a bright prospect in the field of food and pharmaceutics.

Contents

* Corresponding authors.
E-mail addresses: junliu@yzu.edu.cn (J. Liu), chjin@yzu.edu.cn (C. Jin).

https://doi.org/10.1016/j.ijbiomac.2017.10.097

1. Introduction

Phenolic compounds are widely distributed throughout the plant kingdom. As one category of secondary metabolites, phenolic compounds display defensive, growth and development effects on plants [1]. Phenolic compounds are also an essential part of human diet with many valuable bioactivities including antioxidant, anticancer, anti-inflammatory and hepatoprotective activities, which prevent human from many diseases [2,3]. The common structural feature of phenolic compounds is an aromatic ring bearing at least one hydroxyl substituent. A straightforward classification attempts to divide the broad category of phenolics into simple phenols and polyphenols, based exclusively on the number of phenol subunits present. Thus, the term "plant phenolics" encompasses simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, as well as hydrolysable and condensed tannins, lignans and lignins [4]. In general, the bioactivities of phenolic compounds depend on the number and arrangement of hydroxyl groups [5].

In the past decade, many attempts have been made to conjugate phenolic compounds (e.g. phenolic acids and flavonoids) onto polysaccharides by chemical and enzymatic methods [6,7]. The synthesized polysaccharide–polyphenolic conjugates exhibit improved physicochemical properties and biological activities as compared with unmodified polysaccharides and polyphenols [8,9]. Moreover, these conjugates have been demonstrated to have wide applications in food, pharmaceutical and chemical industries [10–12].

As compared with synthesized polysaccharide–polyphenolic conjugates, studies on the naturally occurring polysaccharide–polyphenolic conjugates are still very limited till now. In recent years, several medicinal plants have been demonstrated as valuable resources of naturally occurring polysaccharide–polyphenolic conjugates [13–16]. These conjugates exhibit a great variety of structural and biological properties, depending on the source of medicinal plants, extraction and purification methods [17,18]. What's more, the naturally occurring polysaccharide–polyphenolic conjugates are much safer than chemical synthesized ones. Thus, polysaccharide–polyphenolic conjugates from medicinal plants are certain to have a bright prospect in the field of food and pharmaceutics. For the first time, this article provides recent advances of naturally occurring polysaccharide–polyphenolic conjugates isolated from different medicinal plants. The isolation, purification, structural characterization and biological activities of naturally occurring polysaccharide–polyphenolic conjugates are introduced in details.

2. Extraction and isolation of polysaccharide–polyphenolic conjugates from medicinal plants

Extraction and isolation are the main steps to obtain polysaccharide–polyphenolic conjugates from plant raw materials. Till now, most naturally occurring polysaccharide–polyphenolic conjugates have been extracted and isolated from medicinal plants. However, existing extraction and isolation procedures for plant polysaccharide–polyphenolic conjugates are much similar (Fig. 1). Leaves or flowering parts of medicinal plants are usually selected as the raw materials. The first step is milling, grinding or homogenization of air-dried plant materials. The milled materials are sometimes degreased with methanol or acetone in Soxhlet apparatus. In this process, extractable phenolics can be simultaneously removed [18]. The obtained insoluble part of materials is then subjected to extraction. In general, hot water or alkaline solution can be used for conjugate extraction. For hot water extraction, materials are often refluxed in distilled water at 100 °C for 5 h [13]. For alkaline extraction, materials are usually suspended in 0.1 M NaOH at room temperature for 24 h, and then refluxed at 97 °C for 6 h. The non-extracted part is removed by filtration or centrifugation, whereas the resultant filtrate or supernatant is neutralized by HCl and concentrated, dried to afford the crude extract [19–22].

To isolate polysaccharide–polyphenolic conjugates from the crude extract, sequential liquid–liquid extraction method is commonly adopted. The crude extract is usually first extracted twice with *n*-hexane (water: *n*-hexane = 1:1, v/v) at 69 °C for 6 h followed by extraction three times with diethyl ether (water: diethyl ether = 1:1, v/v) at 34 °C for 6 h. The aqueous fraction is collected and evaporated to obtain the paste-like residue. The residues are further precipitated in methanol at room temperature for 24 h. The obtained precipitate is re-dissolved in distilled water, dialyzed against distilled water and finally dried to afford crude polysaccharide–polyphenolic conjugates [19–22].

The major challenges of existing extraction and isolation methods for polysaccharide–polyphenolic conjugates are the long extraction time, the requirement of costly and high purity organic solvent, the evaporation of a huge amount of solvent, the low extraction selectivity and thermal decomposition of thermal labile compounds [23]. To overcome these limitations, it is supposed that some green extraction techniques, such as ultrasound assisted extraction, enzyme assisted extraction, supercritical fluid extraction and pressurized liquid extraction may be utilized for conjugate extraction in future. At the same time, the influence of green extraction technique on the structures and biological activities of polysaccharide–polyphenolic conjugates should also be considered.

3. Purification of polysaccharide–polyphenolic conjugates from medicinal plants

As polysaccharide–polyphenolic conjugates are polysaccharide–based macromolecules, their purification methods are very similar to those of polysaccharides. In general, anion exchange chromatography and gel filtration chromatography (GPC) are frequently used to purify polysaccharide–polyphenolic conjugates from medicinal plants. The purification of conjugates by anion exchange chromatography is mainly based on different ionic properties of conjugates. Till now, DEAE-Sephadex A-50 and DEAE Sepharose Fast Flow columns have been used to purify polysaccharide–polyphenolic conjugates isolated from *Lythrum salicaria* L. and *Vaccinium vitis-idaea* L. [18,24]. The sample loaded column is often successively eluted with water, gradient sodium chloride solutions or NaOH solutions.

The purification of polysaccharide–polyphenolic conjugates by GPC is based on the molecular size of conjugates. Sephacryl S300 HR and 400HR columns have been used to purify conjugates from *Matricaria chamomilla* L. and *Sanguisorba officinalis* L. The sample loaded column is eluted with 0.1 M NaOH [19,22]. On the other hand, the molecular size distribution of conjugates can be also determined by GPC using a series of dextran standards [18,19]. In the purification process, the conjugate-containing fractions should be monitored by phenol-sulfuric acid method and Folin–Ciocalteu method together [25,26]. The collected conjugate-containing fractions are then dialyzed and freeze-dried to afford the purified conjugates.

It is assumed that other anion exchange columns (e.g. DEAE-cellulose, DEAE Sepharose CL-6B) and GPC columns (e.g. Sephadex and Sepharose) that are frequently used for polysaccharide purification can be also adopted in conjugate purification [27]. On the other hand, the polyphenolic purification methods are sometimes used for conjugate purification. Khoo et al. [13] used the solid phase extraction (SPE) technology to purify polysaccharide–polyphenolic conjugates isolated from *Melastoma malabathricum* L. The crude