International Journal of Biological Macromolecules 101 (2017) 603–611

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

# Isolation, characterization and bioactivities of the polysaccharides from *Dicliptera chinensis* (L.) Juss.

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ARTICLE INFO

*Article history:*
Received 26 August 2016
Received in revised form 10 March 2017
Accepted 19 March 2017
Available online 23 March 2017

*Keywords:*
Anti-aging activity
Antioxidant activity
*Dicliptera chinensis* (L.) Juss.

ABSTRACT

The polysaccharides of *Dicliptera chinensis* (L.) Juss. (DCP-1 and DCP-2) were extracted and isolated using the methods of water extract–ethanol precipitate and sephadex column chromatography and characterized by gel permeation chromatography (GPC), Fourier transform infrared spectrometry (FT-IR) and gas chromatography (GC), respectively. The antioxidant activity of DCPs was evaluated by scavenging activity of DPPH, hydroxyl, superoxide anion and ABTS radical. Moreover, the anti-aging activity of DCP-2 was investigated using an aging model-induced by *D*-galactose (*D*-gal) in mice. The results show that the weight average molecular weight (Mw) of DCP-2 was 2 273 Da with a narrow polydispersity index of 1.01, and it was a heteropolysaccharide and consisted of glucose, galactose, arabinose, rhamnose and mannose with a molar ratio of 3.20:2.54:1.69:1.58:1.00. DCP-2 had stronger antioxidant activity against DPPH, hydroxyl, superoxide anion and ABTS radical, while DCP-1 had hardly any antioxidant activity and DCP had weaker antioxidant activity. Furthermore, DCP-2 can enhance antioxidant capacity and had anti-aging activity against *D*-gal induced aging mice. These results proposed that DCP-2 might be developed as a potential functional food with the activity of anti-aging.



## 1. Introduction

Polysaccharides from natural sources and their derivatives have attracted considerable attention and been considered as the most promising materials in recent years because of their superior properties and extensive application in the fields of pharmaceutics engineering [1,2], food engineering [3], agriculture [4] and others [5,6]. Especially, as natural biopolymers, polysaccharides possess their own biological activities, including antioxidation [7,8], anticancer [9,10], immunoregulation [11,12], antibacterial activity [13], and so on. Therefore, it is necessary to discover and develop the polysaccharides with biological activities as functional food or medicine for the sake of human health.

*Dicliptera chinensis* (L.) Juss. belongs to the family Acanthaceae and is widely distributed in southern China, Bangladesh, northeast India and Vietnam [14], which is used as food as well as traditional Chinese medicine for several purposes, such as detoxification and diuretic action [15]. *Dicliptera chinensis* (L.) Juss. was recorded originally in Herbs Medicine of Lingnan compiled by Xiao Budan in the period of the Republic of China and also included in the Chinese Pharmacopeia (1977). In recent decade the phytochemical studies demonstrated *Dicliptera chinensis* (L.) Juss. contains various chemical constituents, including volatile oils, organic acids, flavonoids, terpenoids, steroids, cerebroside and polysaccharides [16]. In addition, pharmacological researches indicated that the polysaccharide is the main active constituents in *Dicliptera chinensis* (L.) Juss. and has the effect of liver protection and anti-liver fibrosis [17–19]. However, others bioactivities of polysaccharide from *Dicliptera chinensis* (L.) Juss. (DCP) are still unknown.

Hence, as a supplement and extension of previous work, the purpose of the present study was to extract and isolate the DCPs, character their properties and structure, and evaluate the *in vitro*

*Abbreviations:* DCP, polysaccharides of *Dicliptera chinensis* (L.) Juss; GPC, gel permeation chromatography; FT-IR, Fourier transform infrared spectrometry; GC, gas chromatography; *D*-gal, *D*-galactose; DPPH 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); LPO, lipid peroxide; GPX, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; T-SOD, total-superoxide dismutase; T-AOC, total-antioxidant capacity; CAT, catalase; TFA, trifluoroacetic acid; $V_c$, ascorbic acid; CR, congo red.

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http://dx.doi.org/10.1016/j.ijbiomac.2017.03.112
0141-8130/

antioxidant activities and the anti-aging activity using an aging model-induced by *D*-galactose (*D*-gal) in mice.

## 2. Materials and methods

### 2.1. Materials and reagents

*Dicliptera chinensis* (L.) Juss. was purchased from a local commercial market in Guilin, PR China and identified by associate professor Kefeng Zhang. 2, 2- diphenyl-1-picrylhydrazyl (DPPH), pyrogallol, 2,2′-Azino-bis(3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) and trifluoroacetic acid (TFA) were obtained from Sigma Chemical Co. Ascorbic acid ($V_c$) was obtained from Xilong Chemical Co. Ltd. The monosaccharides (mannose, rhamnose, glucose, galactose, arabinose and fructose) and inositol were purchased from Shanghai Yuanye Biotechnology Co. Assay kits of lipid peroxide (LPO), glutathione peroxidase (GPX), superoxide dismutase (SOD), malondialdehyde (MDA), total-superoxide dismutase (T-SOD), total-antioxidant capacity (T-AOC) and catalase (CAT) were purchased from Nanjing Jiangcheng Bioengineering Institute (Nanjing, China). All of other reagents were analytical grade unless stated otherwise.

### 2.2. Extraction and purification of polysaccharides

The dried *Dicliptera chinensis* (L.) Juss. (1000 g) was cut into the section with the length of 3–5 cm and defatted with 95% ethanol in a reflux apparatus, after then extracted with distilled water. The extraction conditions were as follows: temperature of 100 °C, the solid-to-liquid ratio of 1:18 (g/ml), extraction times of 5 and each time 1.5 h. The combined aqueous extracts were concentrated to 2000 ml in a rotary evaporator under reduced pressure at 50 °C. The concentrated solution was precipitated by overnight incubation with ethanol added to a final concentration 90% (v/v) at 4 °C. The precipitate was collected and dissolved in 1600 ml of water, and deproteinized *via* Sevag method (to mix polysaccharide solution with butanol/chloroform (1/5) mixture in a separatory funnel and shake hard, then separate and remove denatured protein by centrifugation). The supernatant was lyophilized (LGJ-12 lyophilizer, Beijing Songyuan Huaxing Technology Development Co., Ltd) to yield the crude polysaccharides (59 g). The crude polysaccharides dissolved in distilled water was decolorized repeatedly with activated carbon (to add little but often activated carbon into polysaccharide solution) until the solution became colorless, and lyophilized to obtain the refined polysaccharides with a product yield of 78% (w/w).

In addition, the refined polysaccharides was loaded on a column (2.5 cm × 100 cm) of Sephadex G-75 equilibrated with distilled water, and was eluted at a flow rate of 0.5 ml/min. In order to detect polysaccharides, a 0.2 ml sample collected from each eluted fraction (2 ml/tube) was mixed with sulfuric acid and phenol to produce color reaction. The fractions with rose color were combined and lyophilized to obtain the light yellow purified polysaccharides. The purified polysaccharides were kept in dryer for further analysis.

### 2.3. Characterization of polysaccharides

#### 2.3.1. Molecular weight (Mw) and its distribution

The Mw and distribution of DCPs were determined by a Waters e2695 gel permeation chromatography (GPC) equipped with a refractive index detector. The 0.2 M $NaNO_3$ in HPLC-grade water was used as eluent with a flow rate of 1 ml/min at 35 °C. The Mw was estimated by reference to a calibration curve made from polyethylene glycols (PEGs) standards.

#### 2.3.2. Fourier transform infrared spectrometry (FT-IR) analysis

FT-IR of DCPs was measured by a Shimadzu IRPrestige-21 spectrophotometer in the frequency range 400–4000 $cm^{-1}$. Samples were dried at 50 °C to constant weight prior to making pellet with KBr powder.

#### 2.3.3. Monosaccharide analysis

The monosaccharide composition of DCP-2 was analyzed by gas chromatography (GC). Briefly, 20 mg polysaccharide was hydrolyzed in 14 ml of 2 M TFA at 100 °C for 10 h under protection of $N_2$. After complete hydrolysis, the digested solution was evaporated under vacuum to dry. 10 mg hydroxylammonium chloride was added to the residue, then acetylation was carried out with 1:1 pyridine-acetic anhydride in water bath at 90 °C for 2 h. The GC operation was performed on an Agilent 7890B instrument using a HP-FFAP column (30 m × 0.53 mm × 1.0 μm, 19095F-123) and flame-ionization detector (FID) using the following conditions: column temperature was programmed from 110 °C (maintained for 1 min) to 150 °C at a rate of 5 °C/min and held for 4 min, then increased to 190 °C at a rate of 5 °C/min and held for 10 min. The rate of $N_2$ carrier gas was 1.0 ml/min, injection temperature was 250 °C. Standards (mannose, rhamnose, glucose, galactose, arabinose and fructose) with inositol as the internal standard were prepared and subjected to GC analysis.

### 2.4. In vitro *antioxidant activity of DCPs*

#### 2.4.1. DPPH radical scavenging activity

The scavenging activity of DCPs against DPPH radicals was determined according to the method [20] with some modifications. The reaction mixture comprised 2 ml of DPPH (0.1 mM in anhydrous ethanol), 1 ml of polysaccharide and 2 ml of anhydrous ethanol. The mixture was incubated at 25 °C for 30 min, and the absorbance of the mixture was determined at 517 nm with $V_c$ as the positive control. The antioxidant activity of the sample was obtained using the equation:

$$\text{Scavenging rate}\,(\%) = [1 - (A_i - A_j)/A_o] \times 100$$

where $A_i$ is the absorbance of DCPs/$V_c$, $A_j$ is the absorbance of background solution and $A_o$ is the absorbance of the control (deionized water instead of sample).

#### 2.4.2. Hydroxyl radical scavenging activity

The scavenging activity of DCPs against hydroxyl radicals was measured according to the method [21] with minor modifications. Briefly, 2 ml of 1.8 mM $FeSO_4$ and 1.5 ml of 1.8 mM salicylic acid (dissolved by alcohol) were added to 1 ml of sample solution at various concentrations. Afterwards, 1 ml of 0.3% (v/v) $H_2O_2$ was added to reaction mixture and incubated at 25 °C for 30 min. The absorbance of the mixture was measured at 520 nm. $V_c$ was used as the positive control. The scavenging activity of the sample toward hydroxyl radicals was calculated using the equation:

$$\text{Scavenging rate}\,(\%) = [1 - (A_i - A_j)/A_o] \times 100$$

where $A_i$ is the absorbance of DCPs/$V_c$, $A_j$ is the absorbance of background solution and $A_o$ is the absorbance of the control (deionized water instead of sample).

#### 2.4.3. Superoxide radical scavenging activity

The scavenging activity of DCPs against superoxide radical was measured according to the method [22] with a minor modification. 0.5 ml of the sample solution with different concentrations was mixed with 5 ml of 50 mM Tris–HCl buffer (pH 8.2). Then 0.2 ml of 6 mM pyrogallic acid was added to the mixture, and the mixture was shaken rapidly and incubated at 25 °C for 5 min. Subsequently,