Food and Chemical Toxicology 50 (2012) 2473–2480

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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

Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*)

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ARTICLE INFO

Article history:
Received 15 August 2011
Accepted 12 October 2011
Available online 19 October 2011

Keywords:
Tea flower
Polysaccharide
Characterization
Antioxidant activity
Hepatoprotective activity
Antitumor activity

ABSTRACT

In the present study, the crude polysaccharides from the flowers of tea plant (*Camellia sinensis*) (TFPS) were prepared with hot water and further fractionated on a DEAE-52 cellulose chromatography to afford three purified fractions of TFPS-1, TFPS-2 and TFPS-3. Then, their preliminary structures, antioxidant and antitumor activities *in vitro* and hepatoprotective activity *in vivo* were investigated. Compared with TFPS-2 and TFPS-3, TFPS-1 had relative higher content of sulfate and relative complicated monosaccharide composition. In addition, TFPS-1 and TFPS-3 showed relative stronger antioxidant activity and inhibitory activity on the growth of human gastric cancer BGC-823 cells. For hepatoprotective activity *in vivo*, we demonstrated that crude TFPS significantly prevented the increase of serum alanine aminotransferase and aspartate aminotransferase levels, reduced the formation of malondialdehyde and enhanced the activities of superoxide dismutase and glutathione peroxidase in carbon tetrachloride-induced liver injury mice. The results suggested that TFPS should be a potent natural polymer with antioxidant, hepatoprotective and antitumor activities.

1. Introduction

Tea, a product made from the leaf and bud of tea plant (*Camellia sinensis* L.), is one of the most widely consumed beverages worldwide. A lot of epidemiological and preclinical studies have demonstrated that drinking tea may reduce the risk of cancer and cardiovascular disease (Bolling et al., 2009; Yang et al., 2009a). Moreover, other biological functions of tea such as anti-allergy, anti-oxidation, anti-inflammation, neuroprotection and anti-obesity have been reported (Dona et al., 2003; Egashira et al., 2004; Fujimura et al., 2002; Khan and Mukhtar, 2007; Rains et al., 2011). These beneficial effects have been partly attributed to its variety of chemical ingredients, including tea catechins, purine alkaloids, theanine and polysaccharides (Bolling et al., 2009; Khan and Mukhtar, 2007; Nie and Xie, 2011).

The flowers of tea plant are also valuable resource. In traditional medicines, tea flowers have been used for deodorization, skin care, cough suppressant and expectorant in China (Yang et al., 2009b). In addition, recent studies have demonstrated that the extract of tea flower had various bioactivities, such as anti-oxidant, gastroprotective and hypoglycemic effects, inhibitory effect on serum triglyceride elevation in olive oil-treated mice, anti-proliferative and apoptotic effects against human breast cancer MCF-7 cells (Joshi et al., 2011; Lin et al., 2003; Yang et al., 2007, 2009b; Way et al., 2009; Yoshikawa et al., 2005, 2008, 2009). These biological effects maybe due to the fact that tea flowers contains similar chemical components as teas, including tea catechins, amino acids, caffeine, carbohydrates, proteins and vitamins (Joshi et al., 2011; Lin et al., 2003; Robin et al., 2011; Wang et al., 2010a;Yang et al., 2007, 2009b). In addition, tea flower contains a variety of functional saponins (Yoshikawa et al., 2005, 2008, 2009). Therefore, tea flowers should have important application value as tea leaves.

Tea polysaccharides, one of the main components of tea extracts, have been demonstrated to have a variety of bioactivities, such as reducing blood sugar level, immunological, anti-radiation, anti-blood coagulation, anti-oxidant, anti-cancer and hypoglycemic effects (Monobe et al., 2008; Nie and Xie, 2011; Wang et al., 2001; Zhou et al., 2007). However, there are few reports on the extraction and bioactivity of polysaccharides from tea flower (TFPS) (Han et al., 2010, 2011a,b; Wang et al., 2010b,c; Wei et al., 2010). Therefore, we report here the preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of TFPS. Firstly, crude TFPS was prepared from tea flowers through extraction by using hot water and ethanol precipitation, and the crude TFPS obtained was further purified through anion-exchange chromatography to afford its purified fractions. Then, the crude TFPS and its purified fractions were characterized by

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doi:10.1016/j.fct.2011.10.047

chemical analysis, gas chromatography (GC) and Fourier transform-infrared spectroscopy (FT-IR). Finally, the antioxidant and anticancer activities *in vitro* and hepatoprotective activity *in vivo* of TFPS were determined.

2. Materials and methods

2.1. Materials

The dried tea flowers of Longjing 43 were provided by the Department of Tea Science, Zhejiang University (Hangzhou, China). The sample was ground into powder using a milling machine and the material that passed through a 40-mesh sieve was kept in sealed polyethylene bags at −20 °C until use. The female Kunming mice were purchased from the Experiment Animal Center of Academy of Military Medical Sciences (Beijing, China). Human gastric cancer BGC-823 cells were obtained from the Cell Bank of Shanghai Institute of Cell Biology (Shanghai, China).

Arabinose, rhamnose, fucose, xylose, galactose, glucose, mannose, ferrozine, nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), reduced nicotinamide adenine dinucleotide (NADH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), bovine serum albumin, 3-(4,5-dimetthylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ) and cisplatin were purchased from Sigma Chemical Co. (MO, USA). Penicillin, streptomycin, fetal bovine serum and RPMI-1640 media were purchased from Gibco/Invitrogen (Grand Island, NY, USA). Assay kits for protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other reagents were of analytical grade.

2.2. Preparation of TFPS

The extraction of crude TFPS was carried out according to the reported method with some modifications (Wang et al., 2010c). Briefly, the powder of tea flowers was pre-extracted two times with 85% aqueous ethanol solution (v/v) in a ratio (material to ethanol solution) of 1:15 (g/mL) at 70 °C for 1 h each, and the supernatant was removed. The resulting residues were extracted three times with distilled water in a ratio (material to water) of material to water 1:20 (g/mL) at 90 °C for 3 h each, and then centrifuged at 5000 rpm for 15 min. The supernatants were combined and concentrated by a rotary evaporator to a proper volume. The resulting concentrate was mixed with three times volume of absolute ethanol, stirred vigorously and kept overnight at 4 °C. The precipitates were then collected by centrifugation at 5000 rpm for 15 min, dissolved in distilled water, dialyzed against distilled water to remove small molecules and lyophilized, affording the crude TFPS.

The crude TFPS was purified by chromatography of DEAE-52 according to our reported method (Qiao et al., 2009a). Briefly, 80 mg of crude TFPS was dissolved in 3.0 mL deionized water, and the solution was filtered through a 0.45 μm membrane filter. Then, the resulting solution of crude TFPS was loaded onto a column (2.6 × 30 cm) of DEAE-52, and the column was stepwise eluted with 0, 0.1, 0.3 and 0.5 M sodium chloride solution at a flow rate of 1.0 mL/min. The eluate was collected automatically (10 mL/tube), and the carbohydrate in each tube was determined by the phenol–sulfuric acid method (Dubois et al., 1956). As a result, three fractions (Fig. 1, F-1, F-2 and F-3) were obtained. They were pooled, concentrated, dialyzed against deionized water and lyophilized, respectively, affording the three purified fractions, named TFPS-1, TFPS-2 and TFPS-3.

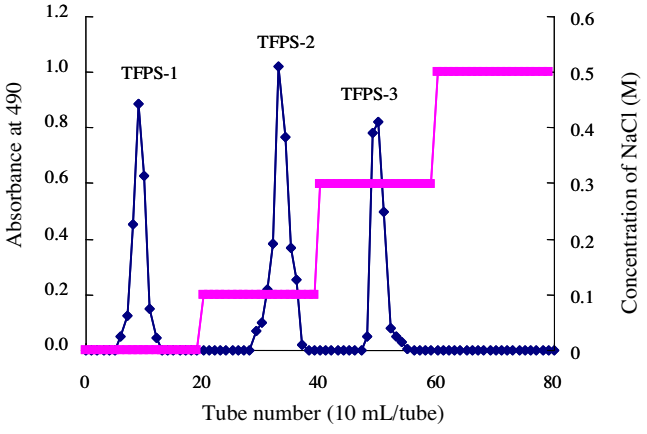

Fig. 1. Stepwise elution curve of crude TFPS on anion-exchange chromatography of DEAE-52 cellulose.

2.3. Preliminary characterization of TFPS

2.3.1. Determination of contents of carbohydrate, sulfuric radical, protein and uronic acid

The contents of carbohydrate in crude TFPS and its purified fractions were determined by phenol–sulfuric acid method using glucose as the standard (Dubois et al., 1956). The content of sulfate radical was determined according to the reported method (Doigson and Price, 1962). The content of protein was determined by the method of Bradford using bovine serum albumin as the standard (Bradford, 1976). The content of uronic acid was determined according to the method of Blumenkrantz and Asboe-Hansen by using D-glucuronic acid as the standard (Blumenkrantz and Asboe-Hansen, 1973).

2.3.2. Analysis of monosaccharide composition of TFPS

The monosaccharide compositions of crude TFPS, TFPS-1, TFPS-2 and TFPS-3 were analyzed by GC according to the reported method with slight modifications (Qiao et al., 2009a). Briefly, the polysaccharide sample was hydrolyzed with 2 mL trifluoroacetic acid (2 M) in an oven at 120 °C for 2 h, The hydrolyzate was repeatedly co-concentrated with methanol to dryness and acetylated by the addition of a mixture of methanol, pyridine and acetic anhydride. In a similar manner, the monosaccharide standards of rhamnose, arabinose, fucose, xylose, mannose, glucose and galactose were acetylated. Then, all the derivatives were analyzed by a 6890 N GC (Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detector and an HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 mm). The operation conditions of GC were as following: flow rates of N_2, H_2 and air were 25, 30 and 400 mL/min, respectively; the temperatures of oven, detector and inlet were 210, 280 and 250 °C, respectively. The injection volume was 1 μL aliquot for each run.

2.3.3. FT-IR spectrometric analysis

The FT-IR spectra of crude TFPS and its purified fractions were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc., MA, USA). The dried sample was ground with potassium bromide powder and pressed into pellet for spectrometric measurement in the frequency range of 4000–400 cm^{-1}.

2.4. Assay of antioxidant activity in vitro of TFPS

2.4.1. Assay of scavenging activity on DPPH free radical

The scavenging activity on DPPH free radical (DPPH·) was measured by using the reported method with some modifications (Luo et al., 2010). Briefly, 0.3 mL DPPH· solution (400 μmol/L in dehydrate alcohol) was added to 3.0 mL of TFPS solution, and the mixture was incubated at 30 °C for 30 min in the dark. Then, the absorbance at 517 nm was measured. Deionized water and ascorbic acid (V_C) were used as the blank and positive control respectively, and the scavenging activity was calculated by the following equation:

$$\text{Scavenging activity}(\%) = [\text{Abs}_0 - (\text{Abs}_1 - \text{Abs}_2)]/\text{Abs}_0 \times 100$$

where Abs_0 is the absorbance of the control (water instead of sample solution), Abs_1 is the absorbance of the sample and Abs_2 is the absorbance of the sample under identical conditions as Abs_1 with dehydrate alcohol instead of DPPH· solution.

2.4.2. Assay of superoxide anion radical scavenging activity

Assay of scavenging activity on superoxide anion radical ($O_2^{\cdot}$) was performed based on the method described by Qiao et al. with some modifications (Qiao et al., 2009b). Each 1.0 mL of sample solution, NBT solution (156 μM of NBT in 0.2 M phosphate buffer, pH 7.4) and NADH solution (468 μM of NADH in 0.2 M phosphate buffer, pH 7.4) were mixed. The reaction was started by adding 1.0 mL of PMS solution (60 μM PMS in 0.2 M phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at room temperature for 5 min, and the absorbance was read at 560 nm against a blank (water and 0.2 M phosphate buffer instead of sample solution and NBT solution, respectively). The scavenging activity on $O_2^{\cdot}$ was calculated using the following equation:

$$\text{Scavenging activity}(\%) = [\text{Abs}_0 - (\text{Abs}_1 - \text{Abs}_2)]/\text{Abs}_0 \times 100$$

where Abs_0 is the absorbance of the control (water instead of sample solution), Abs_1 is the absorbance of the sample solution and Abs_2 is the absorbance of the sample solution under identical conditions as Abs_1 with 0.2 M phosphate buffer instead of NBT solution.

2.4.3. Assay of hydroxyl radical scavenging activity

The hydroxyl radical (·OH) scavenging activity was measured by the method of Jin et al. (1996). The hydroxyl radical was generated in a mixture of 1.0 mL of 0.75 mM 1,10-phenanthroline, 2.0 mL of 0.2 M sodium phosphate buffer (pH 7.4), 1.0 mL of 0.75 mM $FeSO_4$ and 1.0 mL of H_2O_2 (0.01%, v/v). After addition of 1.0 mL sample solution, the mixture was incubated at 37 °C for 30 min. Then, the absorbance of the mixture at 536 nm was measured. Deionized water and V_C were used as the blank and positive control respectively. The scavenging activity on ·OH was calculated by the following equation: