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RADICAL SCAVENGING ACTIVITY OF CRUDE POLYSACCHARIDES FROM *CAMELLIA SINENSIS*

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Abstract – A preparation of crude polysaccharides (TPS) was isolated from *Camellia sinensis* by precipitation and ultrafiltration. TPS1, TPS2, and TPS3 had molecular weights of 240, 21.4, and 2.46 kDa, respectively. The radical scavenging activities of TPS were evaluated by DPPH free radical, hydroxyl radical and superoxide radical scavenging. These results revealed that TPS exhibited strong radical scavenging activity in a concentration-dependent manner. TPS3 with lowest molecular weight showed a higher radical scavenging activity.

Key words: Camellia sinensis, polysaccharides, radical scavenging activity, molecular weight

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INTRODUCTION

Tea (*Camellia sinensis*) has a long history of medicinal use in Asian countries such as China, Japan, India and Thailand which is as ancient as 500 000 years (Chopade et al., 2008). People like tender tea leaves, so the high-grade green tea in the market is usually made from the top two leaves of *Camellia sinensis*. Furthermore, many low-grade coarse green tea leaves are discarded because of their bad taste and color. The chemical composition of tea mainly includes polyphenols (TPP), proteins, polysaccharides (TPS), chlorophyll, and alkaloids (Lee et al., 2009; Müller et al., 2010). Great advances have been made in chemical and bioactive studies of the catechins and polyphenols from tea in recent decades. However, the polysaccharides from tea materials have received much less consideration than TPP. TPS were found to be mostly glycoconjugates in which protein carries, and one or more carbohydrate chains were covalently attached to the polypeptide backbone via N- or O-linkages (Nie and Xie, 2010). The extraction methods and materials of the coarse tea significantly affected the physiochemical and structural features of TPS (Nie and Xie, 2010). In this study, a different molecular weight of low-grade coarse tea polysaccharides (TPS) was isolated by ultrafiltration and their radical scavenging activity was investigated.

MATERIALS AND METHODS

Tea leaves were obtained commercially from the Hebei province of China. 2-Deoxy-D-ribose, nitrotetrazolium blue chloride (NBT), phenazine methosulfate (PMS), linoleic acid, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Aladdin Reagent Int. (Shanghai, China). Nicotinamide adenine dinucleotide (NADH) and Ferrozine were obtained from Sangon Biotech Co. Ltd. (Shanghai, China). Ascorbic acid, trichloroacetic acid (TCA), thiobarbituric acid (TBA) and D-mannitol were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All other reagents and solvents were of analytical purity (AR) grade. All aqueous solutions were prepared by using newly double-distilled water.

General methods

The content of total polysaccharide was determined by the phenol-sulfuric acid method (Dubois et al., 1956). The protein content was measured according to Bradford's method (Bradford, 1976), using bovine serum albumin (BSA) as the standard. Total phenolic content in the TPS was determined according to Khokhar's method (Khokhar and Magnusdottir, 2002).

Isolation of different fractions of crude tea polysaccharides

Dry tea leaves were extracted with 5-times distilled water at 90°C for 2 h; this was repeated twice. The extracts were concentrated and precipitated with 95% ethanol, and then freeze-dried to yield crude tea polysaccharides (TPS). TPS was fractionated into three fractions by means of two ultrafiltration membranes to obtain TPS1, TPS2, and TPS3.

Determination of the molecular weight

The molecular weight of TPS was determined by gel permeation chromatography. Samples were dissolved in 0.02 M phosphate buffer solution and centrifuged at 16 000 r/min for 10 min, and then passed through a 0.45 μm filter. 20 μl of the supernatant was injected into a Shodex SB-804 HQ GPC column (300×8 mm) with a Shodex SB-G guard column (50×6 mm) from Showa Denko K.K. (Tokyo, Japan). The GPC system was maintained at 45 °C and eluted with phosphate buffer solution at a rate of 0.3 ml/min. The molecular weight was calculated by the calibration curve obtained by using various standard dextrans with different molecular weight (T3, T6, T10, T40, T100, T500, and T1000).

Radical scavenging activity - DPPH radical scavenging activity

DPPH free radical scavenging activity of was measured as described by Blois (1958).

Hydroxyl radical scavenging activity

The hydroxyl radical activity was determined by the method of Halliwell et al., (1987).

Superoxide anion scavenging activity

Superoxide anion scavenging activity of CTPS was measured according to Robak and Gryglewski (1988).

RESULTS

Chemical composition and molecular weight of crude tea polysaccharides

The contents of neutral sugar, uronic acid, protein, polyphenol and molecular weight of CTPS are summarized in Table 1. The contents of neutral sugar in TPS, TPS1, TPS2 and TPS3 were determined as 48.90%, 55.70%, 71.60 %, and 59.60%, respectively. TPS2 showed the highest neutral sugar content. The contents of uronic acid in TPS were within the range of 3.95-34.82 %. The molecular weight of TPS1, TPS2, and TPS3 were about 240, 21.4 and 2.46 kDa, respectively.

DPPH radical scavenging activity

The crude tea polysaccharides showed a concentration-dependent DPPH radical scavenging activity (Fig. 1). The scavenging effects of the tea polysaccharides increased with increasing concentration between 25 and 400 μg/mL. The scavenging activity of these fractions was determined as TPS1＞ TPS3＞TPS2＞ TPS. The content of protein and polyphenol was relatively low in TPS1 (2.41% and 4.58%) and TPS2 (1.51% and 4.64%). However, TPS1 and TPS3 showed higher DPPH radical scavenging activity than the fractions with higher polyphenol levels. The EC_{50} values of TPS1, TPS3, TPS2 and TPS were 36, 61, 96 and 119 μg/ml, respectively. These results illustrated that the polysaccharides are the major antioxidant in the crude extracts.

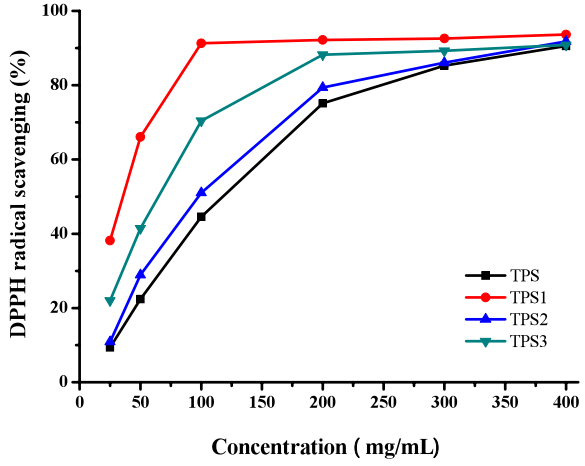

Fig. 1 DPPH radical scavenging activity of crude tea leaves polysaccharides. Each value represents the mean ± SD (n=3)

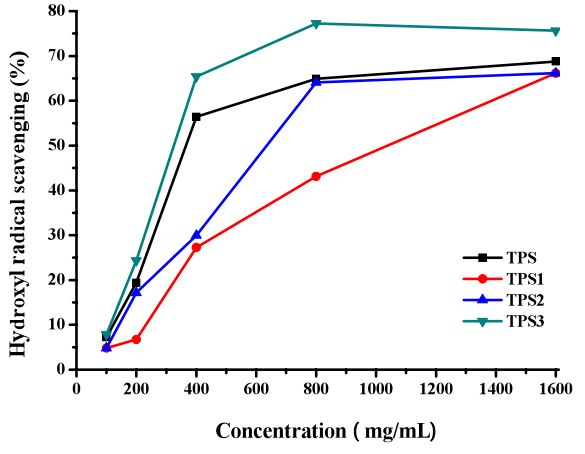

Fig. 2 Hydroxyl radical scavenging activity of crude tea leaves polysaccharides (CTPS). Each value represents the mean ± SD (n=3)

Hydroxyl radical scavenging activity

The inhibitory action of crude tea polysaccharides on deoxyribose degradation gives an indication of the hydroxyl radical scavenging action and iron chelating activity. The crude tea polysaccharides competed with deoxyribose for hydroxyl radicals, and by analysis of the results in terms of a simple competition between CTPS and deoxyribose, a calculation of the rate constant for reaction of CTPS with hydroxyl radicals could be performed

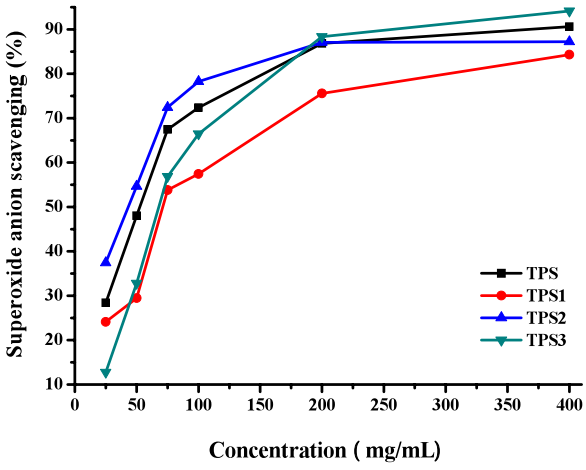

Fig. 3 Superoxide anion scavenging activity of crude tea leaves polysaccharides (CTPS). Each value represents the mean ± SD (n=3).

(Fig. 2). As can seen from the data, the crude tea polysaccharides neutralized the hydroxyl radicals that induced deoxyribose cleavage in a concentration-dependent manner. The scavenging effect of crude tea polysaccharides increased with increasing sample concentration, with EC_{50} values of 322 µg/ml (TPS3), 365 µg/ml (TPS), 632 µg/ml (TPS2), 1040 µg/ml (TPS1), respectively. Hydroxyl radical scavengers suppress hydroxyl radical generation or remove the hydroxyl radicals generated. The crude tea polysaccharides may release a hydrogen proton to react with the hydroxyl radicals causing a decreased rate of hydroxyl radical attack on deoxyribose.

Superoxide anion scavenging activity

Fig. 3 shows the inhibitory effect of crude tea polysaccharides on superoxide radical generation. The scavenging activity of crude tea polysaccharides increased with increasing concentration. The EC_{50} values of TPS, TPS1, TPS2 and TPS3 were about 53, 71, 43, and 67 µg/ml, respectively. Within the concentration range of 25- 400 µg/ml, the superoxide scavenging effects of TPS2 were determined as 37.42 % to 87.21 %. TPS3 showed a stronger scavenging activity (94.13 %) at 400 µg/

Table 1 Composition and molecular weight of crude tea polysaccharide。

Sample	Neutral sugar (wt%)	Uronic acid (%)	Protein (%)	Total phenolic (%)	Molecular weight		
					[a]Mw	[b]Mn	Mw/Mn
TPS	48.90	28.52	4.00	8.86	3597730	3444710	1.04
					516040	371709	1.39
					36619	30478	1.2
					2380	2232	1.07
TPS1	55.70	34.82	2.41	4.58	240218	67268	3.57
TPS2	71.60	10.86	3.75	11.83	21354	13600	1.57
TPS3	59.60	3.95	1.51	4.64	2462	2384	1.03

[a] Mw: Weight-average molecular weight;
[b] Mn: Number-average molecular weight,

ml. There were no significant differences found between the superoxide anion scavenging abilities of TPS2 and TPS.

DISCUSSION

The bioactivities of TPS and their conjugates can also be affected by many factors including chemical components, molecular mass, structure, and conformation. The molecular weights of polysaccharides played an important role in their bioactivity. The TPS from black tea (BTPS) consisted of a high proportion of low molecular weight fractions (3.8-32.7 kD), which was associated with higher bioactivities than those of the TPS from green tea (GIPS, 9.2-251.3 kD) and TPS from oolong tea (OTPS, 5.3-100.9 kD) (Chen et al., 2009). BTPS showed a dose-dependent effect on α-glucosidase inhibitory activity. But GTPS and OTPS hardly inhibited α-glucosidase activity in the same condition (Chen et al., 2009). There was a distinguished difference among these three TPS on the antioxidant activities (Chen et al., 2009). The scavenging rates of GTPS, OTPS and BTPS on DPPH radicals were 47.9%, 23%, and 61.7%, respectively (Chen et al., 2009). Compared with TPC-1 (26.8 kD) and TPC-2 (11.8 kD), TPC-3 (4.2 kD) exhibited the highest antioxidant activities, according to the deoxyribose assay, the photoreduction of NBT assay and the lipid peroxidation inhibition assay (Nie et al., 2008). Here, it was found that TPS3 with lowest molecular weight showed a stronger scavenging activity than TPS2 and TPS1.

The antioxidant abilities of TPS-protein conjugates depend on the protein content (Nie et al., 2008). With increasing protein content, the antioxidant activities of TPS-protein conjugates were enhanced (Nie et al., 2008). The protein may affect the physico-chemical properties of the TPS and hence their bioactivities. The *in vivo* and *in vitro* antioxidant activities of crude tea polysaccharides were found to be superior to tea polysaccharide fraction (Zhou et al., 2007).

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