Composition and Antioxidant Activity of Water-Soluble Polysaccharides from *Tuber indicum*

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ABSTRACT Crude water-soluble Chinese truffle *Tuber indicum* polysaccharide (TIP) was extracted from the fruiting bodies with water and then successively purified by DEAE–cellulose 52 and Sephadex G-100 column chromatography, yielding two major polysaccharide fractions: TIP1-1 and TIP2-1. High-performance gel permeation chromatography analysis showed that the average molecular sizes of TIP1-1 and TIP2-1 were approximately 1.75×10^4 Da and 5.73×10^3 Da, respectively. Monosaccharide component analysis by gas chromatography indicated that TIP1-1 was composed of mannose, glucose, galactose, and rhamannose in the respective molar ratio of 3.93:1.24:0.75:1.26 and that TIP2-1 contained mannose, glucose, and arabinose in the respective molar ratio of 5.27:1.44:0.43. The antioxidant activity analyses revealed that TIP1-1 and TIP2-1 possessed considerable antioxidant activity. Compared with TIP1-1, which has a higher molecular weight and contains no uronic acid, TIP2-1 exhibited a protective effect on PC12 cells injured by H_2O_2 and a higher scavenging activity against free radicals. The relative effects of the lower molecular size, the presence of uronic acid, and the antioxidant activity of TIP2-1 appear to be significant. Accordingly, the Chinese truffle *T. indicum* might serve as an effective antioxidative healthcare food and source of natural antioxidants.

KEY WORDS: • antioxidant activity • polysaccharide composition • *Tuber indicum*

INTRODUCTION

THE TRUFFLE, A TYPE OF HYPOGENOUS mushroom that belongs to the Ascomycota, has long been appreciated as an “edible diamond.” There are more than 100 different truffle species found throughout the world, but the Chinese truffle (*Tuber indicum* Cooke et Massee), widely known as the Asian black truffle, is mainly distributed in the provinces of Sichuan and Yunnan in China. This species has often been exported to Europe as a raw material for flavoring, perfume, and the cosmetic industry because of its unique and characteristic aroma. Also, the Chinese truffle is considered as an edible and medicinal fungus in China because it contains an abundance of proteins, amino acids, carbohydrates, ergosterins, and sterol glycosides.^{1–3} As a traditional folk medicine, the Chinese truffle has been used for adjunctive therapy of gastric cancer by the local national minority people. Previous studies have demonstrated that the *T. indicum* polysaccharides (TIPs) exhibited antitumor and immunoregulation activities.⁴

It is believed that polysaccharides extracted from edible and medicinal mushrooms, such as *Ganoderma lucidum*, *Cordyceps sinensis*, and *Tricholoma matsutake*, possess

numerous beneficial health effects and antioxidant properties due to their free radical scavenging ability and have very few or no toxic side effects compared with synthetic antioxidants.^{5–7} Additionally, phenolic compounds in the desert truffle *Tirmania nivea* also have antioxidant activity against free radicals.⁸ However, until now there have been few reports on the composition and antioxidant activity of the TIPs. Therefore, the aim of the current study was to extract and purify the water-soluble TIPs and to elucidate their composition based on a series of physicochemical and instrumental analyses. The antioxidant activities of the polysaccharides were then tested *in vitro* by evaluating the scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl, and superoxide anions. Furthermore, PC12 cells, a widely used model neuronal cell line, were used to assay the protective effects of polysaccharides against the oxidative stress damage induced by H_2O_2 *in vitro*.

MATERIALS AND METHODS

Compounds

Fresh truffles were collected from soil near the roots of *Quercus* species growing in Panzhihua (altitude, 1,300–1,800 m), Sichuan, China, during the maturing stage of winter truffles in November 2009. They were identified as *T. indicum* Cooke et Massee by the Panzhihua Institute of Agricultural and Forestry Science, Panzhihua, Sichuan, China.

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DEAE-cellulose 52, Sephadex G-100, and T-series dextran were purchased from Amersham Pharmacia Co. (Uppsala, Sweden). Standard monosaccharides of mannose, rhamnose, galactose, xylose, arabinose, fucose, and glucose, as well as trifluoroacetic acid, DPPH, nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), NADH, thiobarbituric acid, dibutylhydroxy toluene (BHT), and methyl thiazolyl tetrazolium (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Extraction, isolation, and purification of polysaccharides

Fresh truffles were cut into pieces, lyophilized, and then ground to fine powder. The dry truffle powder (200 g) was soaked with 4 L of 95% (vol/vol) ethanol for 2 hours at 70°C to remove the phenol pigments and lipids. This procedure was repeated twice.⁹ The residue was dried, followed by extraction with 4 L of distilled water at 80°C (3×6 hours). The pooled filtrate solution was centrifuged at 8,400 g for 10 minutes and then concentrated and dialyzed (molecular weight cutoff, 3,500 Da) (Union Carbide Corp., Houston, TX, USA). The protein liberated from the extract was removed by a Sevag reagent.¹⁰ After removal of the Sevag reagent, the extract was precipitated by washing it four times with ethanol, and the mixture was maintained overnight at 4°C. The precipitate was collected by centrifugation at 8,400 g for 10 minutes and then lyophilized to obtain the crude polysaccharide.

The crude polysaccharide (5 g) was dissolved in distilled water and centrifuged. The supernatant was then subjected to a DEAE-cellulose 52 anion exchange column (2.6×50 cm) equilibrated with distilled water. It was eluted stepwise with 0, 0.1, 0.2, 0.3, 0.5, and 1 M NaCl at a flow rate of 2.0 mL/minute. The fractions were collected with a fraction collector. A molecular sieve Sephadex G-100 column (1.6×50 cm) eluted with distilled water was used to further purify the fractions.¹¹ The carbohydrate content of the samples was determined by the phenol-sulfuric acid method using glucose as a standard.¹² The chromatography profile was generated by OriginPro version 8.0 software (OriginLab Corp., Northampton, MA, USA). The peak with the largest polysaccharide content was collected, dialyzed, and lyophilized.

Component analysis and composition identification

Homogeneity, specific rotation, and average molecular weight identification. The homogeneity of the polysaccharide was assessed by high-performance gel permeation chromatography (HPGPC)¹³ and a specific rotation method.¹⁴ Ethanol was added stepwise to water solutions containing the polysaccharides until the concentrations were 20% and 40% (vol/vol). The polysaccharides were fractionally precipitated, and then an automatic polarimeter (Autopol III, Rudolph Research Analytical, Hackettstown, NJ, USA) was applied to the assay-specific rotation of the polysaccharides precipitated by different ethanol concentrations under the same conditions.

The average molecular weight of the purified polysaccharides was determined by HPGPC.^{15,16} Gel permeation

chromatography was combined with high-performance liquid chromatography using a Millennium 32 workstation (Waters 2695 separations module, Waters Corp., Milford, MA, USA) equipped with an ultrahydrogel column (300×7.8 mm) column and a refractive index detector (model 2410) and elution at a flow rate of 0.6 mL/minute with 1% Na₂SO₄. Standards T-series dextran T-500, T-110, T-70, T-40, and T-10 were used to generate a calibration curve. Empower (Waters Corp.) software was used to calculate average molecular weight.

Monosaccharide composition analysis. The identification and quantification of the monosaccharide units of the polysaccharides were achieved by gas chromatography analysis according to the method of Pan *et al.*¹⁷ and Zhang *et al.*¹⁸ with minor modifications. The sample (10 mg) was completely hydrolyzed with 10 mL of 2 M trifluoroacetic acid in a sealed ampule at 12°C for 6 hours. The excess acid was removed by co-distillation with methyl alcohol after the hydrolysis was completed. The residues (1.0 mg) were silylated with 0.2 mL of anhydrous pyridine, 0.2 mL of hexamethyl-disilazane, and 0.1 mL of trimethyl chlorosilane. The tube was sealed and incubated in a water bath for an additional 20 minutes at 50°C; the standard monosaccharides were derived using the same method. After static cool down, the clear supernatant (2 µL) was loaded into the gas chromatograph for monosaccharide composition analysis. The gas chromatographic analysis was performed on a GC-QP2010 (Shimadzu, Kyoto, Japan) instrument equipped with an Rtx-50 MS capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness; J & W [now Agilent Technologies], Folsom, CA, USA). The temperature of the injector and the interface was set at 250°C. The oven temperature was initially set at 80°C for 3 minutes, increased to 250°C at 10°C/minute, and then held at 250°C for 10 minutes. Helium was used as the carrier gas (head pressure, 72.3 kPa; 80°C; total flow, 44.0 mL/minute). In brief, the molar ratios of the component monosaccharides were calculated using peak area and the relative correction factors.¹⁸

Infrared spectral analysis. The dried polysaccharides were ground with KBr powder for flaking. The infrared spectra were recorded on a Nicolet Nexus 5DXC FT-IR spectrometer (Thermo, Waltham, MA, USA) within the range of 500–4,000 cm⁻¹.

Antioxidant activity analysis

DPPH scavenging activity. The DPPH free radical scavenging activity was measured using the method of Lina *et al.*¹⁹ with minor modifications. The sample (40 mg) was dissolved in 10 mL of distilled water and diluted with gradient 0 (control) to 4 mg/mL. Then, 2 mL of 0.2 mM DPPH in 80% ethanol was added to 1 mL of the different concentrations. The absorbance at 517 nm was recorded after 20 minutes of incubation at 25°C. Simultaneously, BHT (0–4 mg/mL) was used as a positive control. The DPPH scavenging activity in each sample was calculated according to the following equation: DPPH scavenging activity

(%) = $(1 - [A_s - A_{s-blank}]/A_c) \times 100$, where A_c is the absorbance of the control (DPPH solution without sample), A_s is the DPPH solution plus the sample, and $A_{s-blank}$ is the sample without the DPPH solution.

Hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity was determined using the method from Ghiselli *et al.*²⁰ The sample (40 mg) was dissolved in 10 mL of distilled water and diluted with gradient 0 (control) to 4 mg/mL. The sample solution (0.1 mL) was mixed with 0.8 mL of reaction buffer consisting of 0.2 M phosphate buffer (pH 7.4), 1.75 mM deoxyribose, 0.1 mM ferrous ammonium sulfate, and 0.1 mM EDTA and 0.1 mL of 1.0 mM ascorbic acid. H_2O_2 (0.1 mL; 10 mM) was added to the reaction mixture, which was then incubated at 37°C for 10 minutes. Thiobarbituric acid (0.5 mL; 1%) and trichloroacetic acid (1 mL; 2.8%) were added to the reaction mixture, which was brought to a boil for 10 minutes and then cooled on ice. BHT was used as a positive control. The absorbance of the cooled mixture was measured at 532 nm. The following equation was used: Inhibition of deoxyribose degradation activity (%) = $(1 - [A_s - A_{s-blank}]/A_c) \times 100$; all variables in this equation are defined under DPPH scavenging activity.

Superoxide anion scavenging activity. The superoxide anion scavenging activity was measured using the method of Robak and Gryglewski²¹ with minor modifications. The samples (40 mg) were dissolved in 10 mL of distilled water and diluted with gradient 0 (control) to 4 mg/mL. A 1-mL sample solution was mixed with 1 mL of 0.1 M phosphate buffer (pH 7.4) containing 150 μ M NBT, 60 μ M PMS, and 468 μ M NADH. After 5 minutes of incubation at 25°C, the absorbance values at 560 nm were measured. BHT was used as a positive control. The superoxide anion scavenging activity was calculated by the following equation: scavenging activity (%) = $(1 - [A_s - A_{s-blank}]/A_c) \times 100$. The meaning of all variables is described under DPPH scavenging activity.

Protection of PC12 cells from H_2O_2 -induced oxidative stress. Mouse neuronal cell lines (PC12 cells) (American Type Culture Collection, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum, 5% fetal bovine serum, and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2 .

In this study, the protective effects of the polysaccharides against the oxidative stress in PC12 cells were assayed using the method of Li *et al.*²² with modifications. PC12 cells were seeded into 96-well plates at a concentration of 5×10^4 cells/mL using Dulbecco's modified Eagle's medium. After 24 hours, the PC12 cells were pretreated with samples for 2 hours before H_2O_2 (300 mM solution) exposure for 1 hour. After the H_2O_2 was withdrawn, the cells were further incubated in fresh medium for another 6 hours at 37°C. Then, an inverted microscope (ECLIPSE TE2000-E, Nikon, Tokyo, Japan) equipped with a charge-coupled device camera was used to

observe and photograph PC12 cells in 96-well plates. A scale bar was added with charge-coupled device camera software. Subsequently, an MTT stock solution was added to each well, reaching the final concentration of 0.5 mg/mL. After incubation for 4 hours, the supernatants were aspirated to remove the non-transformed MTT. Finally, 150 μ L of dimethyl sulfoxide was added to dissolve the formazan crystals, and the amount of purple formazan was determined by measuring the absorbance at 570 nm with a Universal microplate reader (Bio-Rad, Hercules, CA, USA). Inhibition of cell damage was calculated as: damage inhibitory effect (%) = $([A_s - A]/[A_0 - A]) \times 100\%$, where A_s is the absorbance in the presence of the sample and H_2O_2 , A_0 is the absorbance of the control in the absence of the sample and H_2O_2 , and A is the absorbance in the presence of H_2O_2 .

Statistical analysis

The data were expressed as mean $\pm$ SD values of three replicates. The statistical analysis was carried out using OriginPro version 8.0 software. The half-maximal inhibitory concentration (IC_{50}) values of antioxidant activity were calculated using SPSS version 12.0 software (SPSS Corp., Chicago, IL, USA). One-way analysis of variance was applied for determining the significant differences at $P < .05$.

RESULTS AND DISCUSSION

Isolation and purification of TIP1-1 and TIP2-1

The crude TIP sample was obtained from *T. indicum* fruiting bodies with a yield of 2.91%. Ion-exchange chromatography on a DEAE-cellulose 52 column was used to isolate two fractions—TIP1 and TIP2—from the elution with 0 M and 0.1 M NaCl, respectively (Fig. 1). The two major polysaccharide peaks, TIP1-1 and TIP2-1, were collected and lyophilized (Fig. 2). The total polysaccharide contents of TIP1-1 and TIP2-1 were determined to be 95.4% and 96.1% (wt/wt), respectively.

FIG. 1. Chromatogram of two *T. indicum* polysaccharides (TIPs)—TIP1 and TIP2—on a DEAE-cellulose 52 column eluted with distilled water and the stepwise gradient of NaCl (0.1, 0.2, 0.3, 0.5, and 1 M).

FIG. 2. Chromatograms of (A) TIP1-1 and (B) TIP2-1 isolated from *T. indicum* on a Sephadex G-100 column eluted with distilled water.

Characterization and identification of TIP1-1 and TIP2-1

The HPGPC analyses estimated the average molecular sizes of TIP1-1 and TIP2-1 to be 1.75×10^4 and 5.73×10^3 Da, respectively. The two single and symmetrically sharp peaks of TIP1-1 and TIP2-1 on the chromatogram indicated their homogeneity (Fig. 3). TIP1-1 showed a fixed specific rotation value of $[\alpha]_D^{20} = +28.3^\circ$ (concentration 5×10^{-3} g/mL, in water), and TIP2-1 exhibited one of $[\alpha]_D^{20} = +3.78^\circ$ (concentration 5×10^{-3} g/mL, in water). Their fixed specific rotation values, when they were precipitated by different ethanol concentrations, also indicated their homogeneity.¹⁴

TIP1-1 was composed of mannose, glucose, galactose, and rhamannose in the respective molar ratio of 3.93:1.24:0.75:1.26, and TIP2-1 contained mannose, glucose, and arabinose in the respective molar ratio of 5.27:1.44:0.43.

As shown in Figure 4, the infrared spectrum of the purified TIP1-1 and TIP2-1 fractions revealed no significant differences in the primary absorption intensities between the two fractions. The band between 3,600 and 3,200 cm^{-1} (TIP1-1, 3,431 cm^{-1} ; TIP2-1, 3,409 cm^{-1}) indicated hydroxyl (O-H) stretching. The small band at approximately 2,923 cm^{-1} (TIP1-1, 2,932 cm^{-1} ; TIP2-1,

FIG. 3. Chromatograms of average molecular weight and homogeneity of (A) TIP1-1 and (B) TIP2-1 determined by high-performance gel permeation chromatography. MW, molecular weight.

FIG. 4. Fourier transform infrared spectra of polysaccharides TIP1-1 and TIP2-1.

$2,932\text{ cm}^{-1}$) was attributed to C-H stretching and bending vibrations. The signals at $1,639\text{ cm}^{-1}$ (TIP1-1) and $1,644\text{ cm}^{-1}$ (TIP2-1) were due to the presence of bound water.^{23,24} The absorptions at $1,056\text{ cm}^{-1}$ (TIP1-1) and $1,139\text{ cm}^{-1}$ (TIP2-1) were dominated by ring vibrations that overlapped with C-OH and C-O-C glycosidic stretching vibrations.²⁵ The absorptions at 915 cm^{-1} (TIP1-1) and 914 cm^{-1} (TIP2-1) suggested the presence of pyranoside, whereas the peaks at approximately 885 cm^{-1} (TIP1-1) and 887 cm^{-1} (TIP2-1) were characteristic of β -configuration.⁹ The absorptions at 816 cm^{-1} (TIP1-1) and 815 cm^{-1} (TIP2-1) were the characteristic peaks of mannan.²⁶ However, upon close comparison of the spectra, small differences were observed. The band at approximately $1,750\text{ cm}^{-1}$ in the spectrum of TIP2-1 was attributed to the C=O stretching vibration in the protonated carboxylic acid, which indicated the presence of uronic acid in TIP2-1.²⁷

Antioxidant activity of TIP1-1 and TIP2-1

The literature has demonstrated that polysaccharides with different monosaccharide compositions and molecular weight may exhibit different antioxidant activities, especially in scavenging free radicals.^{28–30} Because excess free radicals are harmful to human health, the free radical scavenging activity of polysaccharides extracted against DPPH, hydroxyl radical, and superoxide anion were evaluated. Moreover, the protective effect of the polysaccharides on the oxidative stress damage induced by H_2O_2 of PC12 cells *in vitro* was also determined.

DPPH is a stable radical, and its model scavenging method is widely used to evaluate antioxidant activity in a relatively short time compared with other strategies. An alcoholic solution containing DPPH free radicals and unpaired electrons exhibits a characteristic purple color, and the discoloration from purple to yellow occurs in the presence of a hydrogen-donating antioxidant because of the single paired electrons. Thus, the DPPH radical scavenging activity of the polysaccharides was determined by measuring the discoloration and absorbance values at 517 nm , and the results were compared with that of BHT. As shown in Figure 5, the polysaccharides exhibited a concentration-dependent antiradical activity. The IC_{50} values of TIP1-1 and TIP2-1 were 1.74 and 1.39 mg/mL , respectively. There was a significant difference between the scavenging activities of TIP1-1 and TIP2-1 on DPPH radicals ($P < .05$). This difference indicated that TIP2-1 was a more effective DPPH radical scavenger than TIP1-1, especially at the high concentration of 4 mg/mL . This high concentration brought the activity of TIP2-1 close to that of the positive control BHT ($P > .05$). However, the scavenging activities of the two samples were lower than that of BHT, the IC_{50} value of which was 0.62 mg/mL .

The hydroxyl radical is one of the most reactive and dangerous reactive oxygen species to human health. The hydroxyl radicals generated through the Fenton reaction in this system were scavenged by TIP1-1 and TIP2-1. As shown in Figure 6, TIP2-1 exhibited a higher scavenging activity against hydroxyl radicals than TIP1-1, whereas both

FIG. 5. Scavenging effects of TIP1-1 and TIP2-1 on 1,1-diphenyl-2-picrylhydrazyl radicals with dibutylhydroxy toluene (BHT) as a positive control. Data are mean $\pm$ SD values ($n=3$).

fractions exhibited concentration-dependent activity. At the concentration of $2\text{--}4\text{ mg/mL}$, activity of BHT was significant higher than that of TIP2-1 ($P < .05$), but there was no significant difference ($P > .05$) between them at concentrations of $0.5\text{--}1.5\text{ mg/mL}$. The IC_{50} values of TIP2-1 and BHT were 1.74 and 1.19 mg/mL , respectively. The IC_{50} value of TIP1-1 was not determinable at this concentration because of its weak activity.

Although superoxide is a relatively weak oxidant, it can decompose and form stronger reactive oxygen species, such as singlet oxygen and hydroxyl radicals, which possess greater oxidative and oleophilic ability than their precursor by initiating lipid peroxidation for a longer time period. In addition, superoxides are known to indirectly induce lipid peroxidation

FIG. 6. Scavenging effects of TIP1-1 and TIP2-1 on hydroxyl radicals with BHT as a positive control. Data are mean $\pm$ SD values ($n=3$).

FIG. 7. Scavenging effects of TIP1-1 and TIP2-1 on superoxide radicals with BHT as a positive control. Data are mean $\pm$ SD values ($n = 3$).

as a result of H_2O_2 formation, thus creating precursors of hydroxyl radicals. In the PMS-NADH-NBT system, superoxide radicals are derived from dissolved oxygen by the PMS-NADH reduction reaction of NBT. The decrease of absorbance at 560 nm indicates the consumption of superoxide radical in the reaction mixture. As shown in Figure 7, the TIPs also exhibited scavenging activity against the superoxide anion in a concentration-dependent manner. The IC_{50} values of TIP1-1 and TIP2-1 were 3.06 and 2.32 mg/mL, respectively, which were higher than that of BHT (1.3 mg/mL). These results indicated that the scavenging activities of TIP1-1 and TIP2-1 were lower than that of BHT.

We determined the protective effects of TIP1-1 and TIP2-1 on oxidative stress damage induced by H_2O_2 in PC12 cells by MTT analysis (Fig. 8). After pretreatment with TIP1-1 and TIP2-1 at 0.25–2 mg/mL, PC12 cells were protected from H_2O_2 (300 mM) injury in a dose-dependent manner. The viability rate of the cells pretreated with TIP2-1 was much higher than that of TIP1-1. As shown in Figure 9 (micrographs of PC12 cells receiving this treatment), almost all the PC12 cells without the pretreatment with polysaccharides were injured by H_2O_2 (Fig. 9A); the decreased viability of the PC12 cells injured by H_2O_2 was partially reversed by pretreatment with TIP1-1 and TIP2-1 in a dose-dependent manner (Fig. 9C–F). However, it was observed that the protective effect of TIP2-1 was higher than that of TIP1-1 at the same concentration. In total, we concluded that TIPs might attenuate the oxidative stress damage of PC12 cells induced by H_2O_2 .

In the antioxidant assay above, the IC_{50} value of TIP2-1 was lower than that of TIP1-1 for both free radical scavenging activity and protective effect on PC12 cells injured by H_2O_2 . Because lower IC_{50} values usually indicate higher activity, TIP2-1 may possess a higher antioxidant activity than TIP1-1 according to our analysis. The mechanism of the

FIG. 8. Protective effect of TIP1-1 and TIP2-1 on the oxidative stress damage induced by H_2O_2 in PC12 cells. Data are mean $\pm$ SD values ($n = 3$).

antioxidant activity of polysaccharides is rather complex. Previous research revealed that this mechanistic complexity was related to their monosaccharide component, molecular weight, structure, and configuration.³¹ In the current study, HPGPC analysis showed that TIP2-1 possesses a lower molecular weight than TIP1-1, and the Fourier transform infrared spectra revealed that both TIP1-1 and TIP2-1 have the characteristic peaks of β -configuration, whereas only TIP2-1 has the indicated carboxyl peak. These data suggest that the antioxidant activity of the polysaccharides may be related to their molecular weight and composition.^{28–30} However, the antioxidant mechanisms of the polysaccharides have not been fully understood so far, and a better comprehension of the relationship between polysaccharide structure such as glucosidic bond and antioxidant activity requires further investigation. Based on the nuclear magnetic resonance and methylation analysis, our work continues to confirm the glucosidic bond in these two polysaccharides.

In addition, the polysaccharides from *T. indicum* exhibited lower free radical scavenging activities compared with the synthetic antioxidant BHT. From the assay of the protective effect against oxidative stress damage in PC12 cells induced by H_2O_2 , the TIPs improved PC12 cell proliferation and provided significant protection. Previous research demonstrated that BHT can induce pulmonary macrophage apoptosis in rats and has potential carcinogenic effects.^{32,33} Considering the side effects of the synthetic antioxidant BHT, we believe polysaccharides from a natural source that exhibits antioxidant activity have more advantages than the synthetic antioxidants.

CONCLUSIONS

In this study, water-soluble polysaccharides TIP1-1 and TIP2-1 were isolated and purified from the fruiting bodies of *T. indicum*. Our results indicated that the antioxidant

FIG. 9. Micrographs showing the protective effect of TIP1-1 and TIP2-1 against oxidative stress damage induced by H_2O_2 in PC12 cells: (A) PC12 cells injured by H_2O_2 (300 mM); (B) normal PC12 cells without H_2O_2 or samples; and PC12 cells injured by H_2O_2 (300 mM) after pretreatment with (C) TIP1-1 (0.5 mg/mL), (D) TIP2-1 (0.5 mg/mL), (E) TIP1-1 (2.0 mg/mL), and (F) TIP2-1 (2.0 mg/mL).

activities were closely related to the molecular weight and uronic acid content. It was also demonstrated that the Chinese truffle *T. indicum* is a healthcare food and a source of natural antioxidants, and further investigation of its antioxidant properties *in vivo* and other studies of the biological activities of these polysaccharides are in progress. Further studies on their application in the food, medical, and cosmetic industries are worth exploration.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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