Comparison in antioxidative and immunomodulatory properties of polysaccharide fractions from *Fuzhuan brick-tea* at different storage periods

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ABSTRACT

*Fuzhuan brick-tea* is a popular Chinese dark tea. It involves a fungal fermentation producing process by *Eurotium cristatum*, which contributes the unique fungal aroma and functions. Polysaccharide fractions extracted from *Fuzhuan brick-tea* (FPS) at different storage stages (production year from 2009 to 2013) were obtained by aqueous extraction. Antioxidant activity to scavenge properties of DPPH and hydroxyl radical were investigated as well as ferric-reducing antioxidant power. Meanwhile, the protective effects on H$_2$O$_2$-induced PC12 cells death and immunomodulatory activities were evaluated. The results demonstrated that FPS from *Fuzhuan brick-tea* had significant radical scavenging activity in a dose-dependent manner. It increased PC12 cell viability with effective growth of 14–94% at the concentration range of 25–200 μg/ml. The immunomodulatory activities were assessed, NO production increased while disposing with 50–400 μg/ml FPS. A tendency was revealed for *Fuzhuan brick-tea* that the best antioxidative effects appear at the third year during storage period. Then the content of neutral carbohydrate, uronic acid and protein were measured to analyse the correlation. Furthermore, results suggested a future direction that antioxidative and immunomodulatory activities of FPS from *Fuzhuan brick-tea* might contribute to nutraceuticals or pharmaceutical industries.

Key words: *Fuzhuan brick-tea*, polysaccharides, antioxidative activity, immunomodulatory activity.

INTRODUCTION

China is one of the major tea producing and exporting countries. According to the manufacturing process, conventional teas are categorized as green tea, yellow tea, white tea, oolong tea, black tea, and dark tea (Wan, 2003). The dark tea is unique for the microbial fermentation process (also called "post-fermentation"), which is considered to be the key factor to form the special flavors and functions of dark tea. *Fuzhuan brick-tea* is one of major brands of dark tea as well as Pu-er tea and Liubao tea (Jin, 2003). Mainly produced in Hunan province of China, *Fuzhuan brick-tea* has been a necessary beverage for the ethnic minorities in the border regions of southern and western China (Chen, 2000).

During the microbial fermentation process, many yellow fungi, which are called "golden flora", grow within the tea leaves under controlled temperature and moisture. The fungus was identified as a mixture of several microorganisms, with *Eurotium spp.* as the dominant one (Mo et al., 2008). The main chemical constituents of *C. sinensis* are similar, mainly classified as total tea polyphenols, catechins, amino acids, polysaccharides, and organic acids. The distinctive post-fermentation process result in the chemical constituents are qualitatively and quantitatively changed and following the characteristic aroma and flavor (Zhang et al., 2013). Several organic acids, including oxalic, L-malic, succinic and ascorbic acid were decreased by fermentation. However, organic acids such as lactic, acetic, and citric acids, which are
fermentation products of beneficial lactic acid bacteria, increased with *Fuzhuan brick-tea* fermentation (Wu, 2010). Compare to green tea (unfermented) and Oolong tea (semi-fermented), the contents of catechins and L-theanine were much less in *Fuzhuan brick-tea*. Although the catechins levels in black tea (fully-fermented tea) were similar to those of Dark teas, the representative catechins oxidation products in black tea, theaflavins and thearubigins were undetectable in *Fuzhuan brick-tea* (Syu, 2008).

The unique post-fermentation technology during dark tea productive process made *Qingzhuang brick-tea* had prominent antioxidant and pancreatic α-amylase inhibiting activities (Cheng et al., 2015). Pu-erh tea polysaccharides can inhibit alpha-glucosidase and have better effect than acarbose at suppressing blood glucose on mice after type 2 diabetes (Deng et al., 2015). The bioactive compounds in *Fuzhuan brick-tea* were indicated as the metabolites due to the process of "fungal fermentation". Studies on microbial fermented teas revealed that the extracts contained natural antimicrobial components which have inhibitive effect on several food-borne spoilage bacteria (Mo et al., 2005). Fermented teas also have gastrointestinal tract regulating effects (Huang et al., 2015). Triterpenoids in *Fuzhuan brick-tea* were assessed on the activities of antibacterial and cytotoxic to pathogenic microbes and some cancer cell lines (Ling et al., 2010). Some secondary metabolites such as flavan-3-ols were regarded as potential bioactive compounds. However, the exact mechanism of the potential tea bioactive compounds associated with health benefits has not been elucidated yet.

Currently, polysaccharides from natural sources have attracted increased attention due to their potential biological functions, especially antioxidant and immunomodulation activities such as scavenging free radicals, inhibiting lipid oxidation, promoting natural killer cells cytotoxicity, and activating macrophages and interleukins. Polysaccharides are main functional components in tea. Tea polysaccharides exhibited strong ability of antioxidant activity in a concentration-dependent manner, the antioxidant and immunological activity were also evaluated (Wang et al., 2013; Zhang et al., 2015). Distinctive manufacturing processes of teas such as the degree of fermentation may affect measurements of assays on antioxidant power. It showed that high fermentation of tea polysaccharides were more potent than the light (Wang et al., 2012). Polysaccharides from tea also have notable immunomodulatory properties. Being lack of immune function of an organism may result in the generation of a tumor, what's more, it impact the prevention of the tumor recovery (Shi et al., 2012). According to the reports, tea polysaccharides enhanced immunization of rats (Nie et al., 2006), furthermore, the immunostimulating activity of crude TPS from immature tea leaves was higher than mature tea leaves.

In this investigation, we prepared *Fuzhuan brick-tea* at different storage stages (production year from 2009 to 2013) from the same producing area. Polysaccharides–rich substances were obtained by aqueous extraction to evaluate the antioxidative effects. Meanwhile, MTT method was applied to assay the protective effects on H2O2-induced PC12 cell death and immunomodulatory properties on RAW264.7.

### MATERIALS AND METHODS

#### Materials and chemicals

The materials of *Fuzhuan brick-tea* were purchased from Hunan Linxiang Yongju tea Co., Ltd, Hunan Province, China. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Gibco Ltd. (Grand Island, NY, USA) and fetal bovine serum (FBS) was from Hyclone (Logan, UT, USA). 1,1’-Diphenyl-2-picrylhydrazyl (DPPH) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were obtained from Sigma Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade as available.

#### Preparation of FPS

As shown in Figure 1, *Fuzhuan brick-tea* was shattered into powder (no less than 50 mesh) with disintegrator and immersed in 95% ethanol (1:4, w/v) for 24 h to remove most of the polyphenols and monosaccharide for a ungeased treatment. After the extracts were filtered, the residues were dried in air and soaked into distilled water (1:20, w/v) at 80°C twice, each for 2 h. The aqueous extracts were filtered and subsequently concentrated to 20% of the initial volume under the negative pressure conditions at 50°C, then precipitated polysaccharide fractions with 4-fold volumes of 95% ethanol. The precipitate formed was collected by centrifugation at 3000 rpm for 20 min. After drying, the refined polysaccharide-rich pellets were completely dissolved in appropriate volume of distilled water and dealt with depigmentation, yielding FPS. The FPS was dialyzed for 3 days against distilled water with dialysis tubing (molecular weight cut-off, 8000 Da) to remove low-molecular weight matters, and then concentrated to obtain the polysaccharide-enriched fractions by vacuum freeze dehydration.

#### Properties of FPS

**Determination of total neutral carbohydrate contents**

Neutral carbohydrate content was determined by the phenol–sulphuric acid method with D-glucose as a standard (Masuko et al., 2005). Briefly, with a slightly modified, 1 ml of sample solution, 1 ml 5% phenol, and 4 ml of
concentrated sulphuric acid were mixed and shaken. The mixture was kept at room temperature for 30 min, then the absorbance was measured at 490 nm.

**Determination of uronic acid contents**

Uronic acid content was measured according to hydroxybiphenyl-sulphuric acid method using galacturonic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973).

**Determination of protein contents**

The protein content was evaluated by Bradford’s method with bovine serum albumin (BSA) as standard (Bradford, 1976).

**Antioxidant activity assay**

**DPPH radical scavenging assay**

The scavenging activity of the DPPH free radical was assayed as described (Cui et al., 2014). Briefly, sample solution (1.0 ml) at various concentrations was added to DPPH (0.2 mM) in 95% ethanol. After vigorously shaken, the mixture was incubated at room temperature for 30 min and the absorbance was determined at 517 nm. The DPPH scavenging rate (R) was calculated as follows:

\[
R \% = \left[ 1 - \frac{Abs\ (\text{sample}) - Abs\ (\text{control})}{Abs\ (\text{blank})} \right] \times 100
\]

where the control solution contains equivalent distilled water instead of DPPH solution, while distilled water instead of sample was used for the blank. All tests were performed in triplicate and the mean of Abs was used in the equation above.

**Hydroxyl radical scavenging assay**

Hydroxyl radical scavenging activity was determined as described by Xiong et al. (2011). A volume of 0.5 ml of sample solutions was mixed with 0.5 ml of salicylic acid-ethanol solution (9.1 mM), 0.5 ml of FeSO₄ solution (9.1 mM) and 3.0 ml of distilled water. The reaction mixture was initiated by the addition of 3.0 ml H₂O₂ (8.8 mM) and measured by absorbance at 510 nm. The hydroxyl radical scavenging rate (R) was calculated as follows:

\[
R \% = \left[ 1 - \frac{Abs\ (\text{sample}) - Abs\ (\text{control})}{Abs\ (\text{blank})} \right] \times 100
\]

Where distilled water instead of H₂O₂ was used for the
control, while distilled water instead of sample was used for the blank. All tests were performed in triplicate and the mean of Abs was used in the equation above.

**Ferric reducing power assay**

The reducing power was determined as described by Yen et al. (2005) with a slight modification. A volume of 0.5 ml indicated concentrations of sample solutions were mixed with 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20 min, and then 0.5 ml of trichloroacetic acid (10%, w/v) was added. After centrifuging at 3000 rpm for 10 min, the supernatant (1 ml) was mixed with deionized water (1 ml) and ferric chloride (0.2 ml, 0.1%). Finally, the absorbance was measured at 700 nm, and the reducing power has a positive correlation with the A700 nm.

**Cell cytotoxicity and protective effects on H2O2-induced PC12 cell death**

These were evaluated in vitro using the MTT assay (Lee et al., 2008). PC12 cells were maintained in DMEM (High Glucose) medium supplemented with 10% fetal bovine serum, 5% heat-inactivated horse serum, 100 U/ml of penicillin and 100 μg/ml streptomycin. Hydrogen peroxide solution (9.8 M) was stored at 4°C prior to use. To check the cell cytotoxicity, cell suspensions were seeded in 96-well plates (1 × 105 /well), and incubated at 37°C for 12 h, and the sample was added. A volume of 20 μl of the MTT stock solution (5 mg/ml) were added into each well after incubating for 6 h, and the plate was further incubated for 4 h. Finally, the medium was removed and DMSO was added (200 μl) to each well to dissolve the formazan. After 10 min, the absorbance was measured at 570 nm in a microtitre plate reader. For protective assay, PC12 cells, after a 30 min pre-incubation with the samples, was subjected to a hydrogen peroxide injury by adding H2O2 with the final concentration 700 μM to the culture media for 6 h (Mu et al., 2012), other procedures were same as above. Assays were performed in quintuplet wells for each sample. Data were expressed as the percent of cell viability compared with control (mean ± SD).

**Determination of immunomodulatory of polysaccharides**

**Assay for nitric oxide (NO) production**

To estimate NO level in RAW264.7 cells, nitrite accumulation was measured by Griess reagent (Gamal-Ekken et al., 2007; Guan et al., 2011; Shi et al., 2012) and used as an indicator of nitric oxide (NO) production in the medium. Briefly, adherent RAW264.7 cells in 96-well plates (1 × 106 cells/well) were stimulated with medium (for the control group), LPS (2 μg/ml) and various concentrations of samples (50, 200, 400 μg/ml) for 24 h at 37°C incubator. After incubation, 100 μl of culture supernatants were mixed with an equal volume of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride, and 2.5% phosphoric acid) in 96-well plates and incubated at 25°C for 10 min. The absorbance at 540 nm was measured on a microplate reader (Perlong DNM-9062, China). Nitrite concentrations in culture supernatants were measured to assess NO production in RAW 264.7 cells. NaNO2 was used as standard to calculate nitrite concentrations.

**RESULTS AND DISCUSSION**

**Extraction yield of FPS**

The crude polysaccharide was isolated from Fuzhuan brick-tea using hot water with yields of 3.9, 3.7, 3.7, 3.3 and 3.2%, respectively, from production year 2009 to production year 2013. The extraction yields increased as the storage periods prolonged. It demonstrated that water-soluble polysaccharides fractions from Fuzhuan brick-tea might increase during storage period.

**In vitro antioxidant activities of FPS**

Burley argued the various mechanisms attributed to antioxidative activities (Diplock, 1997). According to the complexity of the oxidation-antioxidation processes, one test is normally not enough to accurately represent the potential antioxidant activity (Xu et al., 2011). Therefore, we projected the following four assays as further evidences to evaluate the antioxidant activities of the FPS from Fuzhuan brick tea (2009-2013).

**DPPH Radical Scavenging Capability**

The scavenging ability on DPPH is commonly used to evaluate the free radical-scavenging capacity of polysaccharides (Chen et al., 2008; Qiao et al., 2009). The mechanism of scavenging DPPH radical is on the strength of acceptance of hydrogen by the DPPH radical. Accordingly, the capability of antioxidant is subject to the hydrogen donating ability of polysaccharides (Zhao et al., 2014). As shown in Figure 2(A), all five samples of FPS (2009-2013) have the ability of scavenging DPPH radical in a dose-dependent manner at the range of 0.2–2.0 mg/ml. Obvious growth of the scavenging ability was found at the concentration ranges (0.2–0.5 mg/ml) and (1.5–2.0 mg/ml) of the samples. In addition, under the concentration of 2.0
mg/ml, the DPPH radical scavenging activity of FPS-2010, FPS-2011 and FPS-2012 were 41.5, 48.4 and 42.3%, respectively.

For further comparison, FPS at different storage periods were applied under the concentration of 1 and 2 mg/ml. As shown in Figure 2(B), the effect of DPPH scavenging activity increased from the FPS-2013, peaked at FPS-2011, then decreased at the bottom at FPS-2009. The results mentioned above suggested that the FPS-2011 showed stronger activity than the other samples as hydrogen donor, so that they can scavenge DPPH free radical more efficiently. The strongest scavenging ability on DPPH radicals may appear after three-year storage.

**Hydroxyl Radical Scavenging Activity**

The hydroxyl radical is considered to be the most reactive and can induce severe damage to the living organisms (Halliwell et al., 1987; Ke et al., 2009). This radical could be generated by the Fenton reaction in vitro and used to evaluate the hydroxyl radical scavenging ability of natural compounds (Southworth and Voelker, 2003). The mechanism of scavenging hydroxyl radical is related to metal ions transition, thus the ability of scavenging hydroxyl radical depend on chelating metal ions and rendering them inactive (Huang et al., 2005). Removing hydroxyl radical is very important for the protection of living systems. As shown in Figure 3(A), FPS (2009-2013) exhibited the ability to scavenge hydroxyl radicals in dose-dependence. The scavenging effects of polysaccharides of FPS increased stably with increasing concentrations from 2 to 8 mg/ml. Under the concentration of 8 mg/ml, the scavenging rate of FPS-2011 reached up to 60.3% while FPS-2009, FPS-2010, FPS-2012 and FPS-2013 were 56.4, 33.3, 46.5 and 46.9%, respectively. In Figure 3(B), the strongest scavenging ability on hydroxyl radicals appeared at FPS-2011, stored for three years under the concentration of 6 and 8 mg/ml of FPS. FPS-2011 showed remarkably effectiveness than the others at all concentrations tested. It increased from FPS-2013, peaked at FPS-2011, then decrease to FPS-2009.

**EC50 values**

The results above indicated that five polysaccharides-rich fractions for test exhibited a noticeable capacity of
Table 1. EC50 values of FPS (2009-2013) in radical scavenging ability. EC50 value: The effective concentration at which the antioxidant activity was 50%; each value is expressed as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th></th>
<th>FPS-2009</th>
<th>FPS-2010</th>
<th>FPS-2011</th>
<th>FPS-2012</th>
<th>FPS-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH radicals</td>
<td>4.79±0.59</td>
<td>2.50±0.22</td>
<td>1.99±0.04</td>
<td>2.92±0.19</td>
<td>3.92±0.05</td>
</tr>
<tr>
<td>Hydroxyl radicals</td>
<td>15.98±0.25</td>
<td>10.8±0.38</td>
<td>6.75±0.63</td>
<td>6.87±0.33</td>
<td>9.43±0.58</td>
</tr>
</tbody>
</table>

Figure 4. Ferric reducing power of polysaccharides from Fuzhaun brick tea.

scavenging DPPH radicals and hydroxyl radicals. The antioxidant properties assayed herein were summarized in Table 1 and the results were normalized and expressed as EC50 values (mg/ml). FPS-2011, stored for three years, processed the lowest EC50 values in both DPPH radicals and hydroxyl radicals scavenging abilities.

**Ferric reducing power assay**

Different from the precede results on DPPH and hydroxyl radical scavenging, the FRAP (ferric-reducing antioxidant power) assay treats antioxidants contained in the samples as reductants in a redox-linked colorometric reaction, and the value reflects the reducing power of the antioxidants (Muller et al., 2007). The assay is also commonly used for the routine analysis of single antioxidants and total antioxidant activity of plant extracts by measuring Fe3+-Fe2+ conversion. The dose-response curve for the reducing activity of five samples was shown in Figure 4. FPS-2011 showed a stronger reducing activity than other FPSs, indicating the obvious superior Fe3+-Fe2+ conversion ability. At the concentration range from 0.5 to 4 mg/ml, FPS-2011 also manifest the outstanding reducing power, and the tendency in Figure 4 (B) still express superiority of FPS-2011 at the chosen concentration.

**Protective effects on H2O2-induced PC12 cell death**

H2O2 is able to penetrate biological membranes, and plays a radical forming role as an intermediate in the production of more reactive ROS molecules including formation of hydroxyl radical via oxidation of transition metals, and hypochlorous acid by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils, H2O2 has been used in many studies to trigger cell apoptosis (Shui et al., 2006; Tang et al., 2005).

It demonstrates that antioxidants could reduce H2O2-induced PC12 cell apoptotic death through oxidative stress (Shui et al., 2006), the regulation of the endogenous oxidant-antioxidant balance (Xue et al., 2012) and all sorts of alterations of intracellular the death receptor-mediated pathways (Crispo et al., 2010). PC12 cells are cell line originally derived from a pheochromocytoma of the rat adrenal medulla and widely used as a model cell line to assess the protective effects of an antioxidant on H2O2-induced cell death.

MTT assay was applied to detect the affection of the viability of PC12 samples FPS (2009-2013). In Figure 5(A), FPS at the dose of 10–200 μg/ml had no significant toxic effects on PC12 cells. The MTT assay showed that 200–1000 μM of H2O2 could result in the PC12 cell death dramatically, as shown in Figure 5(B). For instance, as shown in Figure 5(C), the incubation of PC12 cells with 700 μM H2O2 for 6 h resulted in a cell viability rate of 37.8% compared to the control. However, when pretreating the cells with three different concentrations (25, 100, 200 μg/ml) of FPS (2009-2013), the cell viability was significantly increased. For FPS-2009, FPS-2010 and FPS-
2011, it had a remarkable growth as the increasing concentrations of samples, and peaked at FPS-2011. Under the concentration of 25-200 μg/ml, the growth is 14-94%, respectively, as shown in Figure 5(C). A significant effect on promoting cell proliferation was obviously observed under the concentration of FPS 200 μg/ml. It also showed the consistent results that storage for three years has the strongest protect effects for PC12. FPS-2011 reached the peak for FPS-2010 and FPS-2009, the effect decrease gradually. Nevertheless, FPS-2012 and FPS-2013 had minor increasing tendency with no outstanding effect, the cell viability was increased by 5-13%.

**Determination of immunoregulation effects**

**Measurement of NO production**

NO was found to be an important mediator of signal transduction in the immune system and participating in the physiology and pathophysiology of many systems (MacMicking et al., 1997; Schepetkin and Quinn, 2006). In recent years, NO was always used as a quantitative index of macrophage activation. Nitrite concentrations in the supernatant of polysaccharide stimulated macrophages were determined as a reflection of NO production. The results of the stimulatory effects of FPS (2009-2013) on the NO production of RAW 264.7 cells were determined by Griess assay. As shown in Figure 6, the five polysaccharide fractions could stimulate macrophages to increase NO production in a dose dependent manner at different concentrations (50, 200, 400 μg/ml) compared with the control. For FPS-2009, FPS-2010, FPS-2011 and FPS-2013, the NO concentration was significantly increased under treatments (50-400 μg/ml), three years storage life has the most significant production. For four and five years of storage lives, it decreased gradually but still higher than the control. The results supposed that FPS may act as stimulators of NO release in macrophages and have the function of activation of macrophages.

**Major chemical contents**

Table 2 shows a summary of the FPS with productive year from 2009 to 2013 of Fuzhuan brick-tea, which contain both neutral carbohydrates and uronic acid with a small amount of proteins relatively after depigmentation procedures. The FPSs were predominantly composed of neutral carbohydrates. Some proteins in FPS might be existing as polysaccharide-protein conjugates. It could still contain polyphenol, lipid in crude polysaccharides according to that less than 100% substance of FPS were obtained in each productive year, some polysaccharides were lost by means of depigmentation. Some water-soluble polysaccharides-rich substances might degraded into smaller components during hot water extraction and subsequently removed during the dialysis, which further leads to decrease in content. The uronic acid content of the extracts from the FPS-2010 to FPS-2013 has significantly growth trend and the carbohydrate content has the
Figure 6. Effects of FPS on the NO production of RAW 264.7 cells. Cells were pretreated with FPS at different concentrations (50, 200, 400 μg/ml) for 24 h. The supernatant nitrite levels were determined using Griess reagent. Experimental values are the mean ± SD values (n = 5). *p < 0.05 compared with the untreated control.

Table 2. Major chemical contents of the FPSs (2009-2013). Each value is expressed as mean ± SD (n = 3).

<table>
<thead>
<tr>
<th>Chemical contents (wt.%)</th>
<th>FPS-2009</th>
<th>FPS-2010</th>
<th>FPS-2011</th>
<th>FPS-2012</th>
<th>FPS-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>41.56±1.61</td>
<td>44.21±1.69</td>
<td>38.20±0.53</td>
<td>26.38±0.20</td>
<td>42.43±2.77</td>
</tr>
<tr>
<td>Protein</td>
<td>5.11±0.29</td>
<td>10.06±0.27</td>
<td>5.25±1.27</td>
<td>3.02±1.46</td>
<td>2.70±1.19</td>
</tr>
<tr>
<td>Uronic acid</td>
<td>29.21±2.22</td>
<td>21.40±2.86</td>
<td>26.81±3.55</td>
<td>28.99±0.51</td>
<td>33.06±2.64</td>
</tr>
</tbody>
</table>

opposite movement, it is feasible that the uronic acid resolved with the storage time result to the growth of carbohydrate content. FPS-2009 did not demonstrate the result which may due to the sudden changes of climate and growth environment, as an example of temperature, humidity and annual rain capacity. Also manual production may be one of influencing factors.

Conclusions

The polysaccharide fractions as functional component in tea had attracted wide attentions. In this study, we obtained carbohydrate-rich fractions FPS (2009-2013) after depigmentation. Colorimetric methods were used to determine the chemical compositions. In addition, the extracted FPS showed significant antioxidant potential including DPPH and hydroxyl radical scavenging activities and ferric-reducing antioxidant power. Moreover, the protective effects of FPS on H2O2-induced PC12 cell death, stimulatory effects on the NO production of RAW 264.7 macrophages were explored. These results illustrated that Fuzhuan brick-tea polysaccharide-rich fractions possess multiple radical scavenging activities in a dose-dependent manner, and also have the potential to protect PC12 cells from H2O2-induced death and to be immunopotentiating agents. Furthermore, studies have shown that for Fuzhuan brick-tea, three years storage life made it had the best antioxidative effects and immunomodulatory activities. From the storage of first year to the third year, the properties increased gradually and decrease from the storage of the fourth year; it had least effect at the fifth year. This study suggested that Fuzhuan brick-tea may have the best quality when it was stored for three years. The Fuzhuan brick-tea polysaccharides were found to be excellent sources of natural antioxidants for health promotion. Further works on the isolation, purification, characterization and additional studies regarding the chemical structure and mechanism of action of the FPS are required to be developed in the food and pharmaceutical
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Conflicts of Interest

The authors declare no conflict of interest.

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