The Impact of Polysaccharides and Flavonoids in Black Fungus on Resistance to Oxidative Stress and X-ray Environmental Radiation

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Abstract. The antioxidant capacity and developmental inhibition to X-ray radiation of polysaccharides and flavonoids in Black Fungus were studied by scavenging ability of free radicals and damage rate of X-ray radiation cells. The results showed that polysaccharide-flavonoid compound with mass ratio of 32.3 had a higher antioxidant effect, and antioxidant complex descended X-ray radiation cell damage rate to 10.06%. The polysaccharides and flavonoids of Black Fungus had effective protection to oxidation and X-ray environment radiation.

1. Introduction
The ionizing radiation from medical and industrial equipment threaten human health during rapid development of science and technology. X-rays ionizing radiation caused oxidative damage to the body, and further damage normal cells, tissues and biological macromolecules. Long-term exposure to small doses of radiation or one-time exposure to large doses of radiation seriously endangered body health.

Natural food ingredients had low toxicity and high efficiency of radiation protection with wide range of sources. The polysaccharides and flavonoids had anti-oxidation and anti-aging activity, anti-tumor and anti-radiation, these dietary antioxidants may protect against oxidative damage induced by radiation sources [1, 2, 3].

In vitro antioxidant properties, free radicals scavenging activities and cells immune regulation of polysaccharides and flavonoids in black fungus were studied to provide a theoretical basis for development of effective radiation protection agents.

2. Materials and methods
2.1. Materials and methods
1,1-Diphenyl-2-trinitrophenylhydrazine (DPPH), Nanjing Aodoforni Biotechnology Co., Ltd.; Porcine Pancreatin and Pepsin, Shenggong Bioengineering Co., Ltd.; Black Fungus, Mudanjiang Longfei Commercial Industry Co., Ltd..

2.2. Instruments and equipments
FW135 high-speed universal crusher, Tianjin Test Instrument Co., Ltd.; GL-16G-II high-speed refrigerated centrifuge, Shanghai Anting Scientific Instrument Factory; 752 UV-Vis Spectrophotometer,
Shanghai Xiepu Instrument Co., Ltd.; KQ-600D CNC Ultrasonic cleaning machine, Kunshan Ultrasonic Instrument Co., Ltd.

2.3. In vitro gastric and intestinal digestion

2 g Black Fungus dry powder was bathed in boiling water with 40 g saline for 15 min, and the sample added 0.25 mL gastric juice (0.2 g pepsin dissolved in 5 mL 0.01 mol/L HCl) to pH to 2.0 with 1 mol/L HCl. The gastric digestive juice adjusted pH to 7.2 with NaHCO3 solution, and added 0.25 mL artificial simulated intestinal juice (4 g porcine pancreatin, 25 g porcine bile salt dissolved in 1 L 0.1 mol/L NaHCO3-Na2CO3 buffer solution). The gastric and intestinal sample were digested in water bath shaker with 100 rpm at 37℃, and were centrifuged with 10,000 rpm at 4℃ for 5 min by different reaction time. The supernatants were Black Fungus antioxidants by gastric and intestinal digestion.

2.4. Antioxidant activity

The scavenging abilities of antioxidants on hydroxyl and DPPH free radicals were determined with IC50 in different concentrations [4, 5]. The synergistic anti-oxidation of polysaccharides and flavonoids were measured with different mass rate by scavenging ability on hydroxyl and DPPH free radicals.

2.5. Cell proliferation ability

Effect of polysaccharides and flavonoids on splenocytes proliferation was exposed under X-ray radiation. The MTT method was used to determine proliferation abilities of polysaccharides and flavonoids in Black Fungus on spleen cells [6].

3. Results and analysis

3.1. Antioxidant activity of Black Fungus during in vitro digestion

The antioxidant substances absorbed in human body mainly attended the mechanism of scavenging free radicals and inhibiting oxidase activity to prevent aging and cardiovascular diseases. The digestive juice of Black Fungus had a 47% scavenging rate of DPPH free radicals after 10 minutes of in vitro gastric digestion (Figure 1.A). The trend of the scavenging effect of Black Fungus antioxidants on DPPH free radicals changed significantly with the prolongation of in vitro gastric digestion time, and the scavenging abilities of DPPH free radicals were different with gastric digestion times.

The scavenging effect of digestive juice of Black Fungus on hydroxyl free radicals gradually increased with treatment time during in vitro gastric digestion. The scavenging rate of Black Fungus antioxidants on hydroxyl free radicals was 1450% at 30 min within in vitro gastric digestion (Figure 1.B). The results showed that Black Fungus had high scavenging ability to DPPH and hydroxyl free radicals during in vitro gastric digestion.
The scavenging rate of digestive juice of Black Fungus on DPPH free radicals was 34% in the initial stage of intestinal digestion after in vitro gastric digestion. The scavenging effect of Black Fungus antioxidants on DPPH free radicals gradually decreased in the prophase of in vitro intestinal digestion, while scavenging rate increased after 30 minutes of in vitro digestion (Figure 2.A). The results showed that Black Fungus had certain scavenging ability on DPPH free radicals by in vitro gastrointestinal digestion.

The scavenging ability of Black Fungus on hydroxyl free radicals gradually increased in digestive juice. The scavenging rate of Black Fungus antioxidants on hydroxyl free radicals was 209% after 90 minutes of in vitro intestinal digestion (Figure 2.B). The results showed that Black Fungus had certain scavenging ability on hydroxyl free radicals by in vitro gastrointestinal digestion.

3.2. Antioxidant activity of Black Fungus polysaccharides

DPPH was widely used to evaluate free radical scavenging activity of antioxidants for its stability. The proton provided by polysaccharides was the main mechanism for scavenging DPPH free radicals. Hydroxyl free radicals was active in reactive oxygen species with short half-life, and derived from peroxide decomposition to danger human health. The IC50 of Black Fungus polysaccharides for scavenging hydroxyl free radicals was 5.583 μg/mL, and value of polysaccharides for scavenging DPPH free radicals was 46.899 μg/mL (Table 1).

| Antioxidant index       | Regression equation | R²   | IC50      |
|-------------------------|---------------------|------|-----------|
| Hydroxyl free radical   | Y=98727x-501.2      | 0.999| 5.583 μg/mL|
| DPPH free radical       | Y=288.7x+36.46      | 0.945| 46.899 μg/mL|

3.3. Antioxidant activity of Black Fungus flavonoids

Black fungus flavonoids regulated body metabolismcan by scavenging free radicals and reduced incidence of cardiovascular and cerebrovascular diseases to prevent chronic diseases. DPPH free radicals reacted on compounds with ability to donate hydrogen, and the solution changed dark purple. The antioxidants treated with DPPH to reduce or disappear absorbance value, and the change had a quantitative relationship with capacity and quantity of antioxidants [7]. The IC50 of black fungus flavonoids for scavenging hydroxyl free radicals was 0.372 mg/mL, and the value of flavonoids for scavenging DPPH free radicals was 1.521 mg/mL (Table 2).

| Antioxidant index       | Regression equation | R²   | IC50      |
|-------------------------|---------------------|------|-----------|
| Hydroxyl free radical   | Y=2515x-886.4       | 1    | 0.372 mg/mL|
| DPPH free radical       | Y=10.72x+33.69      | 0.989| 1.521 mg/mL|
3.4. Synergistic antioxidant of polysaccharides and flavonoids

The scavenging measurement for DPPH free radical was an important method to determine antioxidant capacity of compounds. The scavenging ability of polysaccharides and flavonoids in Black Funguson DPPH free radicals changed continuously with increased concentrations of antioxidant (Figure 3).

![Figure 3. Changes in scavenging rate of DPPH free radicals by different mass ratios of flavonoids and polysaccharides](image)

The scavenging rate of antioxidant to DPPH free radicals reached the highest value of 100% with mass ratio of polysaccharides and flavonoids to 35.7, which was significantly higher than flavonoids, polysaccharides and other compound solutions in scavenging measurement for DPPH free radicals. The results showed that the combination of flavonoids and polysaccharides had a synergistic scavenging effect on DPPH free radicals. The synergistic antioxidant effects had significant difference with combination of polysaccharides and flavonoids, which provided a reference for the screening of composite antioxidants.

![Figure 4. Changes in scavenging rate of hydroxyl free radicals by different mass ratios of polysaccharides and flavonoids](image)

The scavenging rate of antioxidant to hydroxyl free radicals reached the highest value of 100% with mass ratio of polysaccharides and flavonoids to 32.3, and the scavenging value was 83.8% with mass ratio of 44.2 (Figure 4). Hydroxyl radicals existed in the body for a short time of 10-9s, and quickly absorbed electrons to induce lipid peroxidation and electron transfer chain reactions [8].

Polysaccharides and flavonoids had phenolic hydroxyl groups to enhance antioxidant ability, and provided electrons to activate free radical scavenging system. Phenolic radical intermediates reacted with free radicals to stop oxidation reaction, and reduced production of free radicals by inhibiting production of active oxygen, inactivating enzyme activity and chelating metal ions.
3.5. Radiation protection in X-ray

Figure 5. Inhibition of polysaccharide and flavonoids to cell damage in X-ray radiation. A-control group, B-protection group of polysaccharide-flavonoids with mass ratio of 32.3, C- protection group of polysaccharides-flavonoids with mass ratio of 44.2

The cell damage rate in the control group was 19.48%, the rate of polysaccharide flavonoids with mass ratio of 32.3 was 10.06%, and damage rate of antioxidant protection group with mass ratio of 44.2 was 14.63% after culturing for 72 h, respectively (Figure 4). The control group cells suffered obvious radiation damage to disappeared stress response by 72 h treatment, and chromosomal aberrations of the cells died and broked to fragments. The absorbance value of radiation protection group was higher than control group to indicate certain protective effect, and protective capacity of polysaccharide and flavonoids with mass ratio of 32.3 was slightly effective than composite antioxidants with mass ratio of 44.2.

4. Conclusion

The polysaccharides and flavonoids of Black Fungus had scavenging ability to hydroxyl and DPPH free radicals in the process of in vitro simulated digestion, and the combination of compounds had synergistic antioxidant effect. The polysaccharide and flavonoid of Black Fungus had protective effect on X-ray radiation to decrease damage in special work environment.

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