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Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats

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ABSTRACT

Ganoderma lucidum are used as traditional edible and medicinal materials in China. In this study, antioxidant activities of polysaccharides from *G. lucidum* in China were investigated. The influence of *G. lucidum* polysaccharides upon activities of serum antioxidant enzymes and immunity in rats with cervical cancer. The antioxidant activity was measured by $DPPH^-$, O^-, and OH^- free radicals scavenging. Results showed that *G. lucidum* polysaccharides exhibited the higher $DPPH^-$, O^-, and OH^- free radicals scavenging activities. The results still showed that *G. lucidum* polysaccharides could significantly enhance the antioxidant enzyme activities (SOD, CAT and GPx), and reduce levels of IL-1β, IL-6 and TNF-α in rats with cervical cancer.

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1. Introduction

Chinese herbal medicines have been widely used for thousands years for the treatment of fractures and joint diseases. *Ganoderma lucidum* is commonly used in traditional Chinese medicine. In the past, the development of herbal anti-osteoporosis formulas was mainly pursued by scientists in Asian countries, including China, Japan and Korea (Hidaka, Okamoto, Yamada, Kon, & Kimura, 1999; Ke et al., 2009; Xu, Dick, Day, Randall, & Prince, 2003). *G. lucidum* (Fr.) Krast (Polyporaceae), a mushroom-like higher fungus, has been a popular folk and an oriental medicine used to treat many diseases, such as hypertension, hypercholesterolemia, leukemia, and gastric cancer (Paterson, 2006). The polysaccharides isolated from *G. lucidum* have the antitumor activities (Li, Fang, & Zhang, 2007; Paterson, 2006; Zhang, Cui, Cheung, & Wang, 2007). Recently, many new highly oxygenated triterpenes have been isolated from the cultured mycelia of *G. lucidum* (Tang, Gu, & Zhong, 2006; Tang, Liu, Zhao, Wei, & Zhong, 2006; Zhou, Yang, & Yang, 2006), and their new biological functions such as inhibiting the proliferation of lung cancer cell line 95-D, anti-HIV-1, and anti-HIV-1 protease have been reported (El-Mekkawy et al., 1998; Lin, Li, Lee, & Kan, 2003; Tang, Liu et al., 2006). In recent years, the submerged fermentation of *G. lucidum* has received great attention for the efficient production of its valuable metabolites, especially *Ganoderma* polysaccharides (Tang & Zhong, 2002) and ganoderic acid (i.e., GA) (Fang & Zhong, 2002; Tang & Zhong, 2003; Zhong & Tang, 2004).

Carcinoma of the cervix is the second most common cancer to affect females worldwide and is the most common cause of cancer-related death in developing countries (Pisani, Parkin, Bray, & Ferlay, 1999). The purpose of this animal study was to examine in vitro free radical scavenging activities and the preventive effects of *G. lucidum* polysaccharides on oxidative injury and immunity activities in rats with cervical cancer. The free radical scavenging activities of *G. lucidum* polysaccharides was measured. The influence of *G. lucidum* polysaccharides upon activities of serum antioxidant enzymes and immunity in rats with cervical cancer were also evaluated.

2. Materials and method

2.1. Extraction of polysaccharides

The fruiting bodies of *G. lucidum* were purchased from a local medicine shop in Yanchen city, china. Sporocarps were cut into small pieces, dried at 40–50 °C for 48 h and powdered. Polysaccharides were isolated by method of Mizuno (2000) and Pillai, Nair, and Janardhanan (2008) and Yin and Dang (2008) with slight modification. The powdered sporocarps were defatted with petroleum ether and extracted with double distilled water at 80 °C for 8–10 h in several batches. The extract were combined, filtered, and

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concentrated to about one third of the original volume and chilled ethanol about five times the original volume was added and kept at 4 °C for 48 h. The precipitate was collected after centrifugation, redissolved in distilled water and treated with Sevag's reagent (Staub, 1999) several times to remove protein and then dialyzed against deionised water for 48 h at 4 °C. The polysaccharides (crude polysaccharide) were again precipitated with ethanol and the precipitate thus obtained was lyophilized. The crude polysaccharide was dissolved in water and reprecipitated with equal volume of cetyl trimethyl ammonium hydroxide and kept for overnight. The supernatant obtained was precipitated with chilled ethanol. After centrifugation, the precipitate obtained was run through DEAE cellulose column and eluted with deionised water. The precipitate thus obtained was lyophilized to get a light brown powder, (neutral polysaccharide).

2.2. Isolation and purification of Ganoderma lucidum polysaccharides

An aliquot was then applied to an anion-exchange column (5 × 50 cm) of DEAE-Sepharose Fast flow (Pharmacia), and eluted stepwise as two fractions (F1 and F2) (Fig. 1) with 0.1, 0.3, 0.5, 0.7 and 0.9 M NaCl in Tris–HCl buffer (pH 8.5).

2.3. Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was performed on a silica gel plate (5 × 20 cm, Silica gel GF254, Qingdao Haiyang Chemical Co.). An aliquot of each sample was spotted onto the silica gel plate with a developing solvent system of chloroform/methanol (10:1, v/v) or pertroleum ether/ethyl acetate (2:1, v/v). The spots were visualised by spraying the plates with spraying solutions of 1% solution of phenylamine- diphenylamine-phosphate in water. Result from thin-layer chromatography (TLC) indicated that F1 and F2 were both composed of mannose (Fig. 2).

2.4. Free radical scavenging of Ganoderma lucidum polysaccharides

2.4.1. Superoxide anion radical-scavenging activity

Superoxide anion radical-scavenging activity was measured by a non-enzymatic method (Nishikimi, Rao, & Yagi, 1972) modified slightly (Kuda, Hishi, & Maekawa, 2006). Sample solution (0.025 ml) was treated with 0.1 ml of 25 mM phosphate buffer (pH 7.2), 2 mM NADH (0.025 ml) and 0.5 mM NBT (0.025 ml),

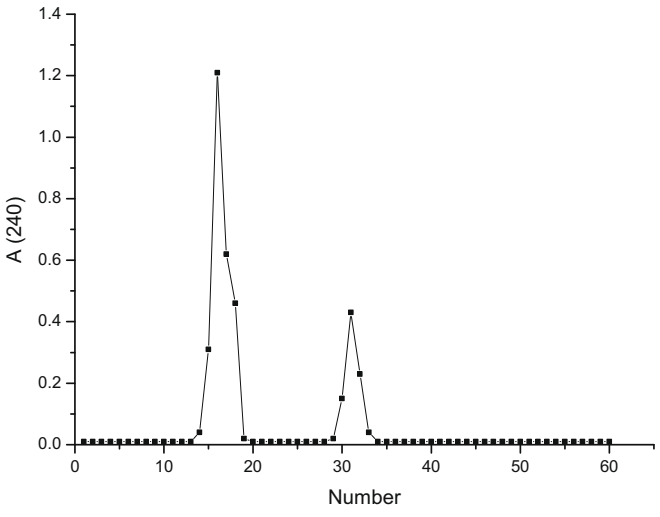

Fig. 1. Isolation and purification of *Ganoderma lucidum* polysaccharides by an anion-exchange column.

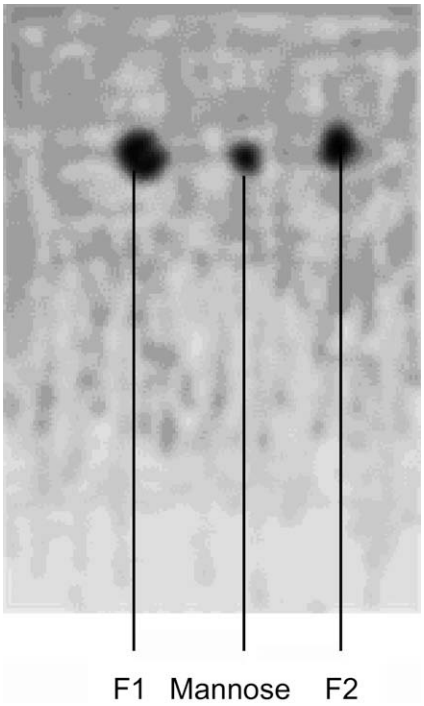

Fig. 2. Thin-layer chromatography.

and absorbance at 560 nm was measured as a blank value. After a 10 min incubation at ambient temperature with 0.025 ml of 0.03 mM PMS, the absorbance was again measured.

2.4.2. Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging activity was assayed according to the method of Lopes, Schulman, and Hermes-Lima (1999). Briefly, the polysaccharides sample was mixed with a solution containing 5 mM 2-deoxyribose, 100 mM H_2O_2, and 20 mM PBS (pH 7.2). Then, reaction was started by the addition of Fe^{2+} (6 μM final concentration) to this mixture. The reaction was carried out for 15 min at room temperature and stopped by adding 4% phosphoric acid (v/v) and 1% thiobarbituric acid (TBA, w/v, in 50 mM NaOH). After boiling for 15 min at 95° C, sample was cooled to room temperature and the absorbance was read at 532 nm.

2.4.3. Measurement of DPPH free radical-scavenging activity

The DPPH free radical-scavenging activities of the *G. lucidum* polysaccharides extract, fractions, and subfractions derived from *Rhodemela confervoides* were measured using the method in a literature report (Yen & Chen, 1995) as well as our previous publication (Duan, Zhang, Li, & Wang, 2006).

2.5. Animal experiment

2.5.1. Treatment of animals

Thirty-two rats of Wistar strain weighing 170–190 g were purchased from the Central Animal House, Suzhou University. The animals were housed in polypropylene cages and maintained under controlled conditions of 12 h light/12 dark cycle and 50% relative humidity at 25–30 °C. The animals were fed pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee, Yanchen 1th Hospital, Suzhou University and the animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals. After a period of 1 week, Twenty-four rats were induced cervical cancer according to the reference (Gao, Shi, Di, & Sun, 2008). Then, the animals with cervical cancer

were divided into three groups of eight rats each and maintained as follows:

Group I (normal control) received the same volume of physiological saline twice daily.
Group II (model control) received the same volume of physiological saline twice daily.
Group III (low dose of polysaccharides treatment) received polysaccharides (dissolved in 2 ml distilled water) at a dosage of 150 mg/kg body weight twice daily.
Group IV (high dose of polysaccharides treatment) received polysaccharides (dissolved in 2 ml distilled water) at a dosage of 300 mg/kg body weight twice daily.

Food and water were fed ad libitum to all groups. At the end of the experimental period of 40 days, body weights of the rats were measured. The rats were sacrificed. Blood was collected in heparinised tubes and plasma was separated. The blood was immediately homogenized in 0.1 M Tris–HCl, pH 7.4. Plasma homogenate was used for various analyses.

2.5.2. Biochemical analysis

SOD, GSH-Px and CAT activities were assessed using commercially available kits (Sigma, America) according to the manufacturer's instructions. One unit of enzyme activity was defined as the amount of protein needed to decrease the reference rate to 50% of maximum inhibition. IL-1β, IL-6 and TNF-α were measured by standard enzyme-linked immunosorbent assay (ELISA) using commercially available BD OptEIA ELISA kits (BD Biosciences, San Diego, CA), as described previously (Stapp, Polis, Sánchez Piñero, 1999).

2.5.3. Statistical analysis

All data were given as means ± standard deviation (SD). Comparisons between the means of various treatment groups were analyzed using Dunnett's *t*-test followed by analysis of variance (ANOVA). $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Superoxide anion radical-scavenging

Superoxide is generated in biological systems during the normal catalytic function of certain enzymes, and in the case of fresh meat, the oxidation of myoglobin (Naguib, 2000). In the present study, we investigated the scavenging or preventive capacity of *G. lucidum* extracts against the superoxide anion free radicals. As illustrated in Fig. 3, the superoxide anion radical scavenging effects of the crude polysaccharides increased with increasing concentration. The highest scavenging activity of the crude extracts was 80.4% at the concentration of 2.2 mg/ml, after that, the value no longer increased (Fig. 3).

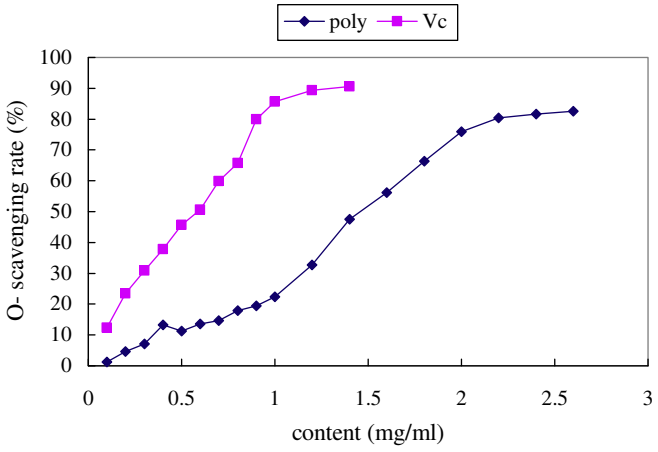

Fig. 3. Superoxide anion radical-scavenging activity of *Ganoderma lucidum* polysaccharides.

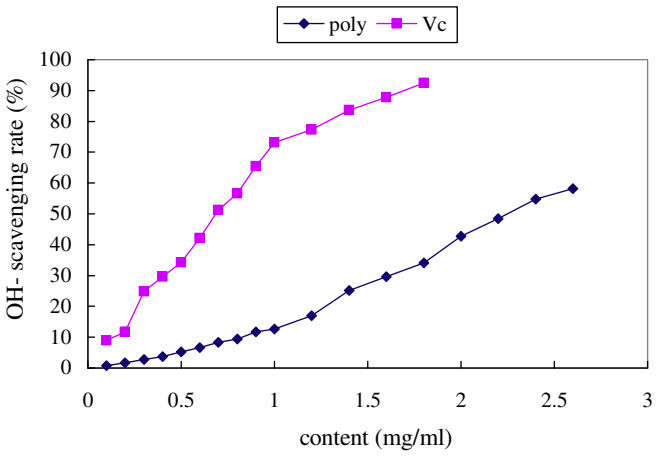

Fig. 4. Hydroxyl radical-scavenging activity of *Ganoderma lucidum* polysaccharides.

3.2. Effect on hydroxyl radical scavenging activities

The hydroxyl radical is one of representative reactive oxygen species generated in the body. In this work, the antioxidant activities of the crude *G. lucidum* polysaccharides were low at the tested concentration range of 0.2–2.6 mg/ml determined by DPPH free radical-scavenging assay. They were 48.4%, 54.8% and 58.2%, respectively at the tested concentration of 2.2, 2.4 and 2.6 mg/ml. The polysaccharides showed lower antioxidant activities than did vitamin C, their scavenging effects increased with increasing concentration (Fig. 4).

3.3. Effect on the DPPH radical-scavenging activity

Because DPPH can be kept indefinitely with little decomposition and because it neither dimerizes nor reacts with oxygen (Yu, Che, Ma, & He, 2009; Yu, Yin, Yang, & Liu, 2009), it proved to be quite useful in a variety of investigations, such as polymerization inhibition or radical chemistry (Wang & Luo, 2007), the determination of antioxidant properties of amines, phenols or natural compounds

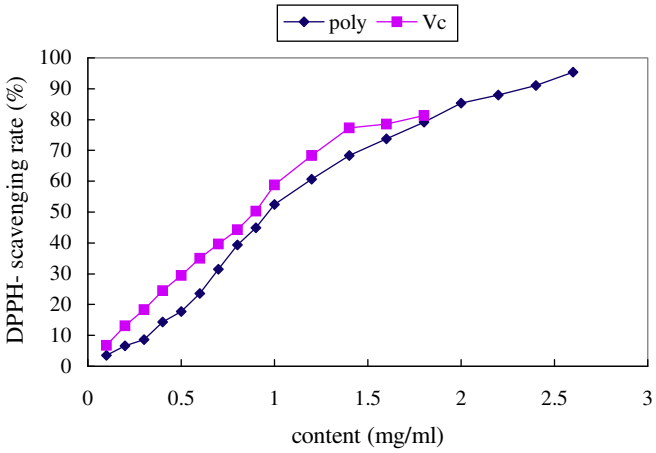

Fig. 5. DPPH radical-scavenging activity of *Ganoderma lucidum* polysaccharides.

Table 1
Effect of *Ganoderma lucidum* polysaccharides on antioxidant enzyme and immunity activities in rats with cervical cancer.

Indexes	Normal control	Model control	*Ganoderma lucidum* polysaccharides (I)	*Ganoderma lucidum* polysaccharides (II)
SOD (U/ml)	143.5 ± 11.8	67.4 ± 3.2[a]	87.8 ± 5.1[b]	115.4 ± 7.3[b]
CAT (U/ml)	15.54 ± 1.13	7.82 ± 0.541[a]	9.48 ± 0.45[b]	13.91 ± 1.17[b]
GPx (U/ml)	25.85 ± 1.43	10.42 ± 0.84[a]	17.34 ± 1.05[b]	26.39 ± 1.33[b]
IL-1β (mg/ml)	1.932 ± 1.107	2.981 ± 0.084[a]	2.471 ± 0.048[b]	2.215 ± 0.131[b]
IL-6 (mg/ml)	2.765 ± 0.093	3.772 ± 0.068[a]	3.472 ± 0.081[b]	3.185 ± 0.092[b]
TNF-α (mg/ml)	1.231 ± 0.089	1.827 ± 0.104[a]	1.613 ± 0.057[b]	1.437 ± 0.071[b]

[a] $P < 0.01$; compared with normal control.
[b] $P < 0.01$, compared with model control.

(vitamins, plant extracts, medicinal drugs) and for inhibiting hemolytic reactions (Yu, Wu, & Niu, 2009). In the present work, the DPPH radical-scavenging assay system was successfully used for the evaluation on the antioxidant activity of the crude extract from *G. lucidum*. The DPPH assay method is based on the reduction of DPPH·, a stable free radical. With the odd electron, the free radical DPPH·gives a maximum absorption at 517 nm by visible spectroscopy (purple colour). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, e.g., a free radical-scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization (yellow colour) is stoichiometric with respect to the number of electrons captured (Blois, 1958).

The values of percent DPPH scavenging activities of *G. lucidum* crude extract were summarised in Fig. 5. These values were compared with those of the well-known antioxidants such as vitamin C. At all concentrations tested, the *G. lucidum* polysaccharides exhibited a dose-dependent DPPH radical-scavenging activity. Detailed analysis for the values listed in Fig. 5, found that, at lower concentrations of 0.2 and 1.8 mg/ml, the *G. lucidum* polysaccharides showed much lower scavenging activity than that of vitamin C, but, when tested at the higher concentration (2–2.6 mg/ml), the *G. lucidum* polysaccharides revealed higher activity to those of vitamin C. This result suggested that the *G. lucidum* polysaccharides is a fairly good scavenger for DPPH radicals.

3.4. Effect of Ganoderma lucidum polysaccharides on antioxidant enzyme and immunity activities in rats with cervical cancer

The antioxidant enzymes activities (SOD, CAT and GPx) was significantly decreased in serum of rats with cervical cancer (model group) compared to control ($p < 0.01$), but administration of *G. lucidum* polysaccharides dose-dependently significantly enhanced antioxidant enzymes activities (SOD, CAT and GPx) in the serum of rats fed with polysaccharides (III and IV groups) ($p < 0.01$) (Table 1) compared to model group. In addition, as shown in Table 1, levels of IL-1β, IL-6 and TNF-α (Table 1) was also significantly higher in serum of rats with cervical cancer (model group) compared to the control group ($p < 0.01$). Administration of *G. lucidum* polysaccharides was dose-dependently significantly decreased in the serum of rats fed with polysaccharides (III and IV groups) ($p < 0.01$) (Table 1) compared to model group.

4. Conclusions

The *G. lucidum* polysaccharides in China exhibited the antioxidant potent to some degree in in vitro assays, and could enhance the antioxidant enzyme activities (SOD, CAT and GPx), and reduce levels of IL-1β, IL-6 and TNF-α in rats with cervical cancer. Therefore, the potency of the polysaccharides compounds could provide a scientific basis for some of the health benefits for cervical cancer diseases. Further studies are needed to investigate the physiological and pharmacological properties of the *G. lucidum* polysaccharides, which is of potential research and development value in the field of pharmaceutical and functional foods.

References

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181*, 1199–1200.

Duan, X. J., Zhang, W. W., Li, X. M., & Wang, B. G. (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chemistry, 95*, 37–43.

El-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., et al. (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. *Phytochemistry, 49*, 1651–1657.

Fang, Q. H., & Zhong, J. J. (2002). Two-stage culture process for improved production of ganoderic acid by liquid fermentation of higher fungus *Ganoderma lucidum*. *Biotechnology Progress, 18*, 51–54.

Gao, G., Shi, T., Di, J., & Sun, J. (2008). Establishment of cervical cancer transplanted carcinoma models in rats. *Journal of Clinical Rehabilitative Tissue Engineering Research, 12*, 5494–5498.

Hidaka, S., Okamoto, Y., Yamada, Y., Kon, Y., & Kimura, T. (1999). A Japanese herbal medicine, Chujo–to, has a beneficial effect on osteoporosis in rats. *Phytotherapy Research, 13*, 14–19.

Ke, C. L., Qiao, D. L., Gan, D., Sun, Y., Ye, H., & Zeng, X. X. (2009). Antioxidant acitivity in vitro and in vivo of the capsule polysaccharides from *Streptococcus equi* subsp. Zooepidemicus. *Carbohydrate Polymers*, *75*, 677–682.

Kuda, T., Hishi, T., & Maekawa, S. (2006). Antioxidant properties of dried product of 'haba-nori', an edible brown alga, *Petalonia binghamiae* (*J. Agaradh*) Vinogradova. *Food Chemistry, 98*, 545–550.

Li, Y. Q., Fang, L., & Zhang, K. C. (2007). Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *Ganoderma lucidum*. *Carbohydrate Polymers, 68*, 323–328.

Lin, S. B., Li, C. H., Lee, S. S., & Kan, L. S. (2003). Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. *Life Science, 72*, 2381–2390.

Lopes, G. K. B., Schulman, H. M., & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. *Biochimica et Biophysica Acta, 1472*, 142–152.

Mizuno, T. (2000). Development of an antitumor biological response modifier from *Phellinus linteus* (Berk et curt) Teng (Aphyllophoromycetidae). *International Journal of Medicinal Mushrooms, 2*, 21–33.

Naguib, Y. M. A. (2000). A fluorimetric method or measurement of oxygen radical-scavenging activity of water soluble antioxidants. *Analytical Biochemistry, 284*, 93–96.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochemical and Biophysical Research Communications, 46*, 849–854.

Paterson, R. R. M. (2006). *Ganoderma*—a therapeutic fungal biofactory. *Phytochemistry, 67*, 1985–2001.

Pillai, T. G., Nair, C. K. K., & Janardhanan, K. K. (2008). Polysaccharides isolated from *Ganoderma lucidum* occurring in Southern parts of India, protects radiation induced damages both in vitro and in vivo. *Environmental Toxicology and Pharmacology, 26*, 80–85.

Pisani, P., Parkin, D. M., Bray, F., & Ferlay, J. (1999). Estimates of the worldwide mortality from 25 cancers in 1990. *International Journal of Cancer, 83*, 18–29.

Stapp, P., Polis, G. A., & Sánchez Piñero, F. (1999). Stable isotopes reveal strong marine and El Niño effects on island food webs. *Nature, 401*, 467–469.

Staub, A. M. (1999). Removal of protein. Sevag method. *Methods in Carbohydrate Chemistry, 5*, 5–6.

Tang, W., Gu, T. Y., & Zhong, J. J. (2006). Separation of targeted ganoderic acids from *Ganoderma lucidum* by reversed phase liquid chromatography with ultraviolet and mass spectrometry detection. *Biochemical Engineering Journal, 32*, 205–210.

Tang, W., Liu, J. W., Zhao, W. M., Wei, D. Z., & Zhong, J. J. (2006). Ganoderic acid T from *Ganoderma lucidum* mycelia induces mitochondria mediated apoptosis in lung cancer cells. *Life Science, 80*, 205–211.

Tang, Y. J., & Zhong, J. J. (2002). Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid. *Enzyme and Microbial Technology, 31*, 20–28.

Tang, Y. J., & Zhong, J. J. (2003). Scale-up of a liquid static culture process for hyperproduction of ganoderic acid by the medicinal mushroom *Ganoderma lucidum*. *Biotechnology Progress, 19*, 1842–1846.

Wang, Z. J., & Luo, D. H. (2007). Antioxidant activities of different fractions of polysaccharide purified from *Gynostemma pentaphyllum* Makino. *Carbohydrate Polymers, 68*, 54–58.

Xu, M., Dick, I. M., Day, R., Randall, D., & Prince, R. L. (2003). Effects of a herbal extract on the bone density, strength and markers of bone turnover of mature ovariectomized rats. *American Journal of Chinese Medicine, 31*, 87–101.

Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry, 43*, 27–37.

Yin, G. H., & Dang, Y. L. (2008). Optimization of extraction technology of the *Lycium barbarum* polysaccharides by Box–Behnken statistical design. *Carbohydrate Polymers, 74*, 603–610.

Yu, Z. H., Che, J., Ma, X., & He, J. M. (2009). Effect of *Aloe vera* polysaccharides on immunity and antioxidant activities in oral ulcer animal models. *Carbohydrate Polymers, 75*, 307–311.

Yu, D.-H., Wu, J.-M., & Niu, A.-J. (2009). Health-promoting effect of LBP and healthy Qigong exercise on physiological functions in old subjects. *Carbohydrate Polymers, 75*, 312–316.

Yu, Z. H., Yin, L. H., Yang, Q., & Liu, Y. (2009). Effect of *Lentinus edodes* polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. *Carbohydrate Polymers, 75*, 115–118.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology, 18*, 4–19.

Zhong, J. J., & Tang, Y. J. (2004). Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites. *Advances in Biochemical Engineering/Biotechnology, 87*, 25–59.

Zhou, Y. Q., Yang, X. T., & Yang, Q. Y. (2006). Recent advances on triterpenes from *Ganoderma* mushroom. *Food Reviews International, 22*, 259–273.