African Journal of Microbiology Research Vol. 6(2), pp. 302-307, 16 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1312
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Promotion and inhibition to biomass and polysaccharides production of *Ganoderma Lucidum* by *Sophora flavescens* extract

Yan-Qun Li[1]* and Zhi-Cong Zhi[2]

[1]College of Food Science and Technology, Guangdong Ocean University, Huguangyan Dong, Zhanjiang, 524088, Guangdong Province, China.
[2]Guangzhou No.5 Middle School, 32 Nancun Road, Guangzhou, 510220, Guangdong Province, China.

Accepted 29 November, 2011

***Sophora flavescens* water extract of 11 g/l promoted *Ganoderma lucidum* biomass and extracellular polysaccharides production to increase at 14.5 and 2.4 g/l from 10.5 and 1.2 g/l respectively. However, 40 g/l of this extract stopped the fungus growing. The extract increased the pH buffer capacity and decreased the viscosity of the culture broth. Different extracts of *S. flavescens* obtained using solvents n-hexane, chloroform, ethyl acetate, n-butanol and water, successively were evaluated on their effects on *G. lucidum* biomass and polysaccharides production. The ethyl acetate extract was found to decrease both the biomass and the extracellular polysaccharides production by about 50%. However, the n-butanol extract promoted *G. lucidum* to increase the extracellular polysaccharides production from 1.15 to 2.05 g/l.**

Key words: *Ganoderma lucidum*, *Sophora flavescens*, polysaccharide, submerged culture.

INTRODUCTION

Ganoderma lucidum (Fr.) Krast (Polyporaceae), a basidiomycete is a famous traditional Chinese medicine which has been used as tonic and invigorating medicine, usually accompanied with other medicinal herbs such as *Sophorae flavescentis* in the recipes for clearing “heat” and “toxicity” in traditional Chinese medical practices. Recent pharmacological studies have proven that *G. lucidum* extract shows immune regulation (Zhang et al., 2002), anti-tumor (Li et al., 2011; Liu and Zhong, 2011; Wang et al., 1997), anti-virus (El-Mekkawy et al., 1998; Kim et al., 2000) and hepato-protection activities (Baek et al., 1999). The added herbs enhanced the medicinal effects of the recipes (Li et al., 2006). Polysaccharides (Li et al., 2007; Sanodiya et al., 2009; Zhang et al., 2010) and and ganoderic acids (Fatmawati et al., 2010; Jiang et al., 2011; Liu and Zhong, 2011; Shi et al., 2010) are the key actives in *G. lucidum*. Increasing cell biomass production and active ingredient concentrations can enhance the medicinal efficiency of *G. lucidum*. Efforts have been made at submerged culture conditions to increase the production of mycelia and polysaccharides (Huang et al., 2009; Liu and Zhang, 2007; Yang and Liau, 1998; Yang et al., 2009, 2004). In our previous studies, water extracts of some traditional herb medicines have been used to complement the media to enhance and improve the medical efficiency of *G. lucidum* submerged culture broth (Li et al., 2006, 2007).

Some traditional herbs, including *S. flavescens*, not only enhanced the medicinal efficiency but also promoted the mycelia growth and polysaccharides production. The current study investigates *S. flavescent* promotion of *G. lucidum* mycelia growth and polysaccharides production.

MATERIALS AND METHODS

Preparation of *Sophora flavescens* water extract

The dried sliced root of *Sophora flavescens* was purchased from Guangzhou Pharmaceutical Holding Limited (Guangzhou, China).

*Corresponding author. E-mail: liyq2004@126.com. Tel: 86-759-2396026. Fax: 86-759-2396028.

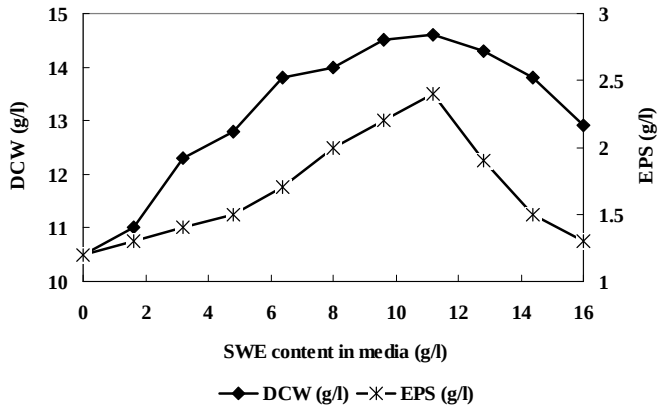


Figure 1. Dry cell weight (DCW) and extracellular polysaccharides (EPS) of *Ganoderma lucidum* cultured in the media containing different quantity of *Sophora flavescens* water extract (SWE). DCWs and EPSs were measured at 120 h of cultivation time. The culture media were basic medium plus SWE.

1 kg of *S. flavescens* root was extracted with 6 L water at boiling temperature for 30 min. The decoction was collected and centrifuged. The supernatant was concentrated with rotary vacuum evaporator and then lyophilized and obtained 102 g of *S. flavescens* water extract (SWE).

Fractions of *Sophora flavescens* extracts

900 g dried sliced *S. flavescens* root was extracted with 2000 ml petroleum ether (boiling range 35 to 60℃) following Soxhlet's method for 4 h. The extract solution was collected and evaporated to remove organic solvent to obtain the *S. flavescens* correspond-dent organic solvent extract. The root was then put into Soxhlet's extractor again after removing the residue solvent to extract with new solvent (chloroform, ethyl acetate, n-botanol and ethanol successively) according to aforementioned process. Finally, the root was extracted with 3000 ml boiling water. Each extract was prepared for addition into media.

Strain and cultivation

Ganoderma lucidum #GIM 5.259 was purchased from Guangdong Culture Collection Center (Guangzhou, Guangdong Province, China). The basic culture medium contained (g/l): glucose, 30; peptone, 4; yeast extract, 2; KH_2PO_4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.75; vitamin B_1, 0.01. *G. lucidum* was cultured in a 5 l bioreactor containing 3 l media at 30℃.

Determination of dry cell weights

The culture broth was centrifuged at 3400 g to obtain mycelia. The mycelia was dried at 80℃ and then weighted for the dry cell weight (DCW).

Extracellular polysaccharide (EPS) measurement

The culture broth was centrifuged at 3400 g, and the supernatant was concentrated in a rotary vacuum evaporator and precipitated by adding 4 cm^3 95% (v/v) ethanol. The precipitate was collected by centrifugation at 10000 g and re-dissolved with water to measure polysaccharides with the phenol/sulfuric acid assay.

Viscosity measurement

Viscosity measurement was carried out in a Brookfield LVDV-α^+ viscometer (Brookfield, USA) at 30 ± 0.1℃. Culture br oths were centrifuged at 3400 g to remove cells and insoluble particles, and then the cell free broths were used to test the rheological data. The shear stresses and the correspondent shear rates were fitted with the following model to obtain the rheological parameters:

$$\tau = k\gamma^n$$

Where τ is the shear stress (N/m^2), γ is the shear rate (1/s), k is a consistency index (Pa.s) and n is a flow behavior index. K and n were calculated from a serial data of τ and γ. The apparent viscosities (η_a) were calculated with $\eta_a = k\gamma^{n-1}$. As the stirring speed of the bioreactor was 250 rpm, the shear rate was estimated as 26.17 (1/s) that is 2π × 250/60. So, the apparent viscosities were calculated as $\eta_a = 26.17^{n-1}k \times 1000$ (mPa.s).

Determination of pH buffer capacity

The buffer capacity of media solution or culture broth was measured by titration with HCl solution. 50 ml media solution or culture broth was titrated with a 0.1 M HCl solution and the pH was monitored by a pH meter. The volume of HCl solution cost in titration and the corresponding pH were recorded to evaluate the pH buffer capacities.

RESULTS

Effect of *S. flavescens* water extract on cell groth of *G. lucidum*

Figure 1 shows that the *S. flavescens* water extract promoted biomass growth and extracellular polysaccha-rides production. However, the SWE concentration should be regulated (approximately 11 g/l) such that both DCW and EPS are maximized for product production. SWE concentrations above 11 g/L favour high EPS at the cost of DCW. We suspected the presence of trace in the SWE which present effects at high SWE. Subsequent trials demonstrated that when SWE content was added up to 32 g/l final concentration in the medium, *G. lucidum* grew slowly after a 20-day lag-phase. With 40 g/l SWE, *G. lucidum* did not grow within a 40-day observation period. Figure 2 showed the time profile of DCW and EPS when *G. lucidum* was cultured in a medium containing 11 g/l SWE or in a basic medium. As Figure 2 showed, *G. lucidum* had a longer growth phase of about 108 h in the SWE containing medium compared with the 84 h of growth phase in the basic medium and as a result a higher biomass production was obtained in the SWE containing medium. Figure 2 also showed that EPS were synthesized during cultivation period by the mycelia but not the polysaccharides residue from the extract.

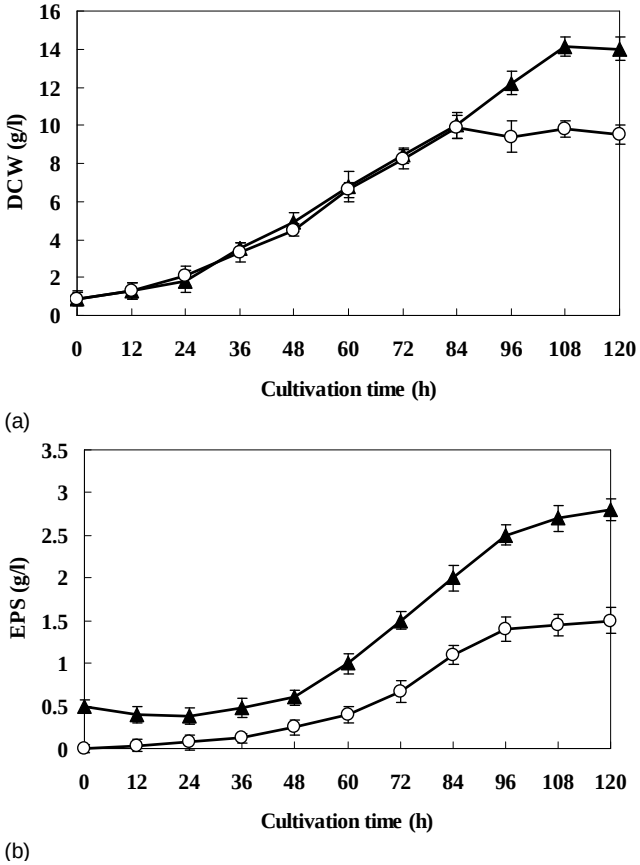


Figure 2. The dry cell weight (DCW) and extracellular polysaccharides (EPS) of *Ganoderma lucidum*. ▲, the medium was composed of basic medium and 11 g/l of *Sophora flavescens* water extract (SWE) and ○ basic medium as control.

S. flavescens water extract enhanced the pH buffer capacity of medium

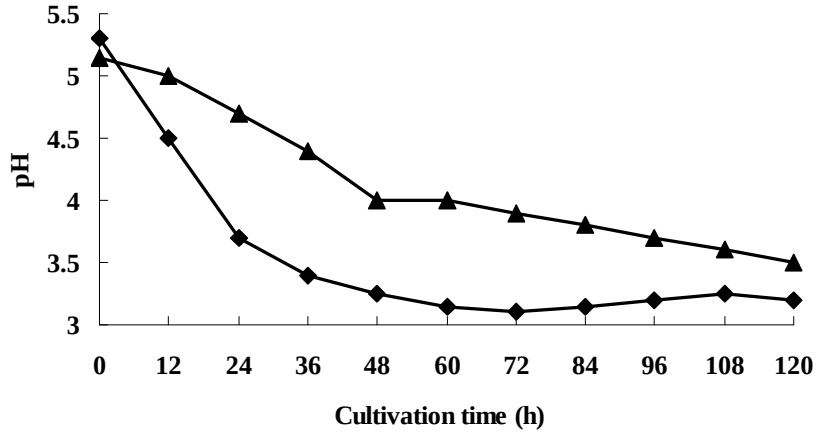
With the growth of *G. lucidum*, the pH of culture broth declined quickly, however, SWE slowed the decline as illustrated by Figure 3a. Usually, low pH is inhibitory to *G. lucidum* mycelia growth. The SWE buffering effect was beneficial to *G. lucidum* cell growth and could be regarded as one of the promoting factors. Increasing the pH buffer capacity of medium should be one of the reasons for delaying culture broth pH decline. As shown in Figure 3b, an additional 10 ml of HCl is required to acidify SWE containing medium. Obviously, the SWE increased the pH buffer capacity of the medium.

Apparent viscosity of culture broth

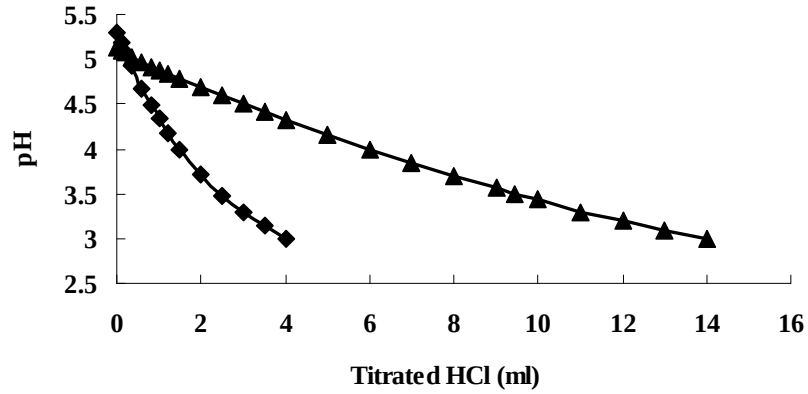
The apparent viscosities (η_a) of the culture broths were measured once a day throughout 5 days of cultivation. As of day two, the control broth was much more viscous than the SWE containing broth (Figure 4); notably the control broth had near double apparent viscosity of the SWE containing broth at the fifth day. Lower viscosity broth is advantage to the mass transfer and may ultimately benefit in cell growth and metabolites production. Therefore, SWE mediated reduction of medium viscosity further promotes growth.

The effects of different extract fractions

The extraction fractions obtained with n-hexane, chloroform, ethyl acetate, n-butanol and water successively were added into *G. lucidum* cultivation media separately. The control medium was only a basic medium without any *S. flavescens* extract. Figure 5 showed the DCW and EPS of the culture broths at the 5 day of cultivation. In the results, the ethyl acetate extract significantly inhibited the cell biomass and EPS production when the chloroform extract impacted slightly both productions and the n-hexane extract did not show any impact. Interestingly, the



(a)



(b)

Figure 3. The effects of *Sophora flavescens* water extract (SWE) on media pH. pH of culture broth in different cultivation time (a) and pH change of media with titration by 0.1 mol/l HCl (b); and ◆ basic medium as control; ▲, the medium composed of basic medium and 11 g/l of SWE.

n-butanol fraction increased EPS production significantly without any change of the cell biomass production. The aqueous extract increased much both the mycelia growth and polysaccharides production. The results indicated that the ethyl acetate extract contained inhibiting factors both on biomass growth and EPS production and the n-butanol extract contained stimulating factors only on the EPS production. *S. flavescens* contain a lot of alkaloids (1 to 2.5% in dry root), some of which have the ability to inhibit fungi cell growth and their metabolism. Ethyl acetate might have extracted the majority of alkaloids from the root such that the extract exhibited the result of magnifying the inhibitory effects of the crude decoction. The increase in DCW resulting from aqueous extract addition is commonly expected. The aqueous extract should have contained aqua soluble nutrients such as sugars and amino acids which could have promoted *G. lucidum* cell reproduction and extracellular polysaccharides synthesis. However, the promoting effect of n-butanol extract on extracellular polysaccharides production was most interesting.

It is valuable to further investigate which compound(s) in the n-butanol extract could have the notable promoting effect on extracellular polysaccharides production.

DISCUSSION

In order to increase the mycelia and medicinal metabolites (mainly polysaccharides and ganoderic acids) production, cultivation processing strategies and condition optimization have been examined in detail (Hsieh et al., 2006; Tang et al., 2009, 2011; Zhang and Tang, 2008). Moreover, some researchers tried to add special compounds or extracts from animals or plants intothe media. Ren et al. (2010) added methyl jasmonate into *G. lucidum* culture and increased ganoderic acids productivity by 45%. Yang et al. (2004) tried ethanol,

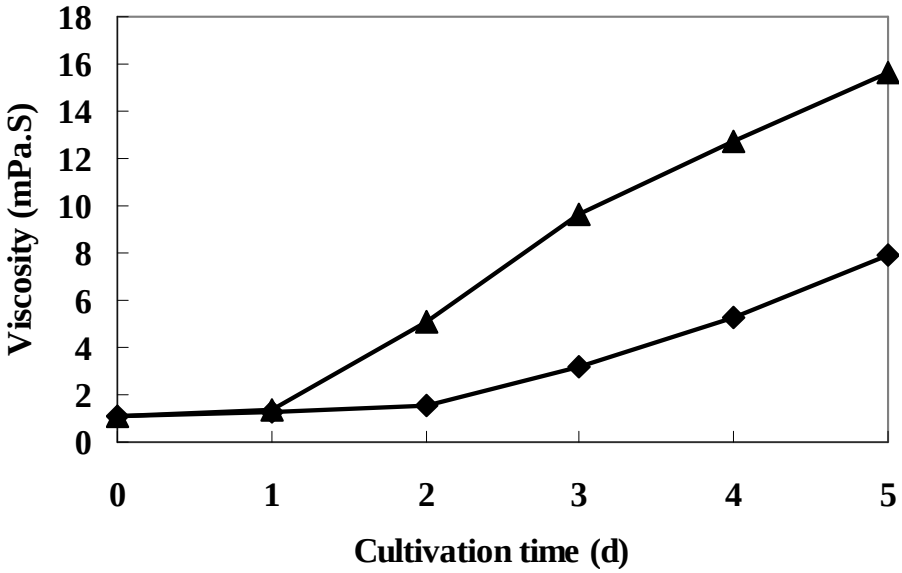


Figure 4. The apparent viscosities of the *Ganoderma lucidum* culture broth in different medium and different cultivation time. ▲, basic medium as control; ◆, the medium composed of basic medium and 11 g/l *Sophora flavescens* water extract (SWE).

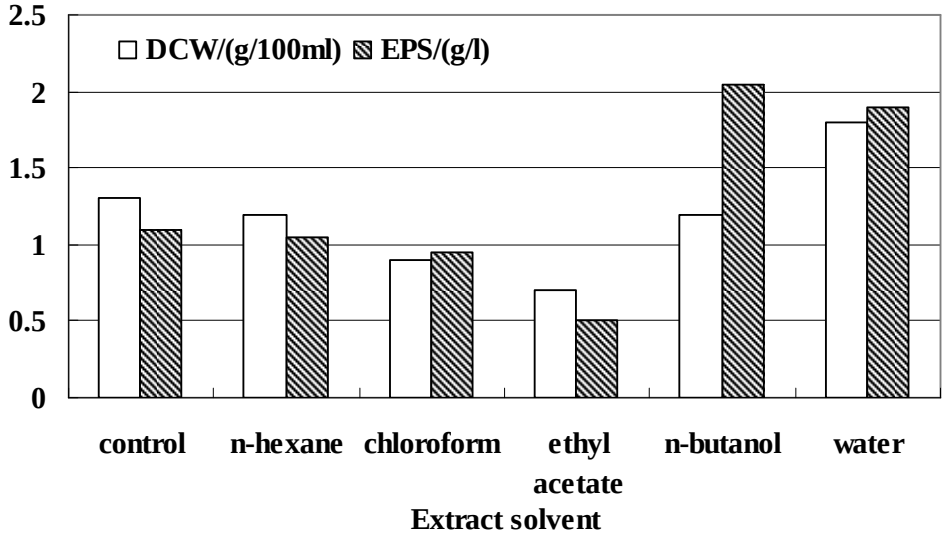


Figure 5. Effects of extracts by organic solvents and water on the dry cell weight (DCW) and extracellular polysaccharides (EPS) production.

Yang et al. (2000) investigated some fatty acids, Liu and Zhang (2007) tried ethyl acetate extract of *Eupolyphaga sinensis* (a medicinal insect) and Zhu et al. (2008) tested polysaccharides isolated from a fungus, *Tuber aestivum vittad* to increase biomass and extracellular polysaccharides production of *G. lucidum*. However, the mechanisms are still unknown. Moreover, the studies on the effects of herbal medicine extracts were rarely reported. In this current study, it was shown that appropriate amount of water extract of *S. flavescens* can promote *G. lucidum* cell growth and extracellular polysaccharides production, although too much extract could severely inhibit *G. lucidum* growth. The *S. flavescens* extract can decrease culture broth viscosity that is beneficial for mass transfer, leading to improved oxygen transfer coefficient so that the mycelia growth

was improved finally. During growth, the pH of broth with the *S. flavescens* water extract declined slower than that of control to give a relatively gentle environment for *G. lucidum* growth. Solvent fractionation and addition during cultivation of *G. lucidum* proved that there were promoting and inhibiting factors in *S. flavescens* to *G. lucidum* cell growth and extracellular polysaccharides production.

The inhibition substances mainly exist in the ethyl acetate extract and the promoting factors of extracellular production exist in n-butanol extract in additional to the water soluble sugars and amino acids. In summary, the results in this current study indicate that *S. flavescens* extract can promote *G. lucidum* cell growth and extracellular polysaccharides production through decreasing culture broth viscosity, increasing broth pH buffer capacity and providing nutrients and some special promoting substances. Moreover, *S. flavescens* contains some inhibiting substances which can severely depress *G. lucidum* cell growth and polysaccharides production.

ACKNOWLEDGEMENT

This work was financially supported by the initiative funding from the Guangdong Ocean University. The authors thank Mr. Mark Horsman very much for refining the manuscript language.

REFERENCES

Baek SJ, Jung WT, Kim DY, Cho Ii KJ (1999). G009 Hepatoprotectant immunostimulant. Drug. Future, 24:1068-1071.

El-Mekkawy S, Meselhy MR, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N, Shimotohno K, Kawahata T, Otake T (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. Phytochem., 49:1651-1657.

Fatmawati S, Shimizu K, Kondo R (2010). Ganoderic acid Df, a new triterpenoid with aldose reductase inhibitory activity from the fruiting body of *Ganoderma lucidum*. Fitoterapia., 81:1033-1036.

Hsieh C, Tseng MH, Liu CJ (2006). Production of polysaccharides from *Ganoderma lucidum* (CCRC 36041) under limitations of nutrients. Enzym. Microb. Technol., 38:109-117.

Huang HC, Chen CI, Hung CN, Liu YC (2009). Experimental analysis of the oil addition effect on mycelia and polysaccharide productions in *Ganoderma lucidum* submerged culture. Bioprocess. Biosyst. Eng., 32:217-224.

Jiang Z, Jin T, Gao F, Liu J, Zhong J, Zhao H (2011). Effects of Ganoderic acid Me on inhibiting multidrug resistance and inducing apoptosis in multidrug resistant colon cancer cells. Process. Biochem., 46:1307-1314.

Kim YS, Eo SK, Oh KW, Lee CK, Han SS (2000). Antiherpetic activities of acidic protein bound polysacchride isolated from Ganoderma lucidum alone and in combinations with interferons. J. Ethnopharmacol., 72:451-458.

Li W, Nie S, Chen Y, Wang Y, Li C, Xie M (2011). Enhancement of cyclophosphamide- induced antitumor effect by a novel polysaccharide from ganoderma atrum in sarcoma 180-bearing mice. J. Agric. Food Chem., 59:3707-3716.

Li Y, Yang Y, Fang L, Zhang Z, Jin J, Zhang K (2006). Anti-hepatitis activities in the broth of *Ganoderma lucidum* supplemented with a Chinese herbal medicine. Am. J. Chinese Med., 34:341-349.

Li YQ, Fang L, Zhang KC (2007). Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *Ganoderma lucidum*. Carbohydr. Polym., 68:323-328.

Liu GQ, Zhang KC (2007). Enhancement of polysaccharides production in *Ganoderma lucidum* by the addition of ethyl acetate extracts from Eupolyphaga sinensis and Catharsius molossus. Appl. Microbiol. Biotechnol., 74:572-577.

Liu RM, Zhong JJ (2011). Ganoderic acid Mf and S induce mitochondria mediated apoptosis in human cervical carcinoma HeLa cells. Phytomedic., 18:349-355.

Ren A, Qin L, Shi L, Dong X, Mu DS, Li YX, Zhao MW (2010). Methyl jasmonate induces ganoderic acid biosynthesis in the basidiomycetous fungus *Ganoderma lucidum*. Bioresource Technol., 101:6785–6790.

Sanodiya BS, Thakur GS, Baghel RK, Prasad GBKS, Bisen PS (2009). *Ganoderma lucidum*: A potent pharmacological macrofungus. Current. Pharm. Biotechnol., 10:717-742.

Shi L, Ren A, Mu D, Zhao M (2010). Current progress in the study on biosynthesis and regulation of ganoderic acids. Appl. Microbiol. Biotechnol., 88:1243-1251.

Tang YJ, Zhang W, Zhong JJ (2009). Performance analyses of a pH-shift and DOT-shift integrated fed-batch fermentation process for the production of ganoderic acid and *Ganoderma* polysaccharides by medicinal mushroom *Ganoderma lucidum*. Bioresource Technol. 100:1852–1859.

Tang YJ, Zhang W, Liu RS, Zhu LW, Zhong JJ (2011). Scale-up study on the fed-batch fermentation of *Ganoderma lucidum* for the hyperproduction of ganoderic acid and *Ganoderma* polysaccharides. Process. Biochem., 46: 404-408.

Wang SY, Hsu ML, Hsu HC, Tzeng CH, Lee SS, Shiao MS, Ho CK (1997). The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. Intern. J. Cancer, 70:699-705.

Yang FC, Ke YF, Kuo SS (2000). Effect of fatty acids on the mycelial growth and polysaccharide formation by Ganoderma lucidum in shake flask cultures. Enzym. Microb. Technol., 27:295-301.

Yang FC, Liau CB (1998). The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged cultures. Process. Biochem., 33:547-553.

Yang FC, Yang MJ, Cheng SH (2009). A novel method to enhance the mycelia production of *Ganoderma lucidum* in submerged cultures by polymer additives and agitation strategies. J. Taiwan Institut. Chem. Eng., 40:148-154.

Yang HL, Wu TX, Zhang KC (2004). Enhancement of mycelial growth and polysaccharide production in *Ganoderma lucidum* (the Chinese medicinal fungus, 'Lingzhi') by the addition of ethanol. Biotechnol. Lett., 26: 841-844.

Zhang J, Tang Q, Zhou C, Jia W, Da Silva L, Nguyen LD, Reutter W, Fan H (2010). GLIS, a bioactive proteoglycan fraction from *Ganoderma lucidum*, displays anti-tumour activity by increasing both humoral and cellular immune response. Life Sci., 87:628-637.

Zhang J, Tang Q, Zimmerman-Kordmann M, Reutter W, Fan H (2002). Activation of B lymphocytes by GLIS, a bioactive proteoglycan from *Ganoderma lucidum*. Life Sci., 71:623-638.

Zhang W, Tang YJ (2008). A novel three-stage light irradiation strategy in the submerged fermentation of medicinal mushroom *Ganoderma lucidum* for the efficient production of ganoderic acid and *Ganoderma* polysaccharides. Biotechnol. Prog., 24:1249-1261.

Zhu LW, Zhong JJ, and Tang YJ (2008). Significance of fungal elicitors on the production of ganoderic acid and *Ganoderma* polysaccharides by the submerged culture of medicinal mushroom *Ganoderma lucidum*. Process. Biochem., 43:1359–1370.