

Full Length Research Paper

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

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***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 *Sophora flavescens* 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 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. flavescens* promotion of *G. lucidum* mycelia growth and polysaccharides production.

## MATERIALS AND METHODS

### Preparation of *S. flavescens* water extract

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

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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 *S. flavescens* extracts

900 g dried sliced *S. flavescens* root was extracted with 2000 ml petroleum ether (boiling range 35 to 60°C) following Soxhlet's method for 4 h. The extract solution was collected and evaporated to remove organic solvent to obtain the *S. flavescens* correspondent 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-butanol 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;  $\text{KH}_2\text{PO}_4$ , 1.5;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.75; vitamin B<sub>1</sub>, 0.01. *G. lucidum* was cultured in a 5 L bioreactor containing 3 l media at 30°C.

#### Determination of dry cell weights

The culture broth was centrifuged at 3400 g to obtain mycelia. The mycelia was dried at 80°C 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<sup>3</sup> 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-II viscometer (Brookfield, USA) at 30 ± 0.1°C. Culture broths 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  $\tau$  is the shear stress (N/m<sup>2</sup>),  $\gamma$  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  $\tau$  and  $\gamma$ . The apparent viscosities ( $\eta_a$ ) 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  $2\pi \times 250/60$ . 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 growth of *G. lucidum*

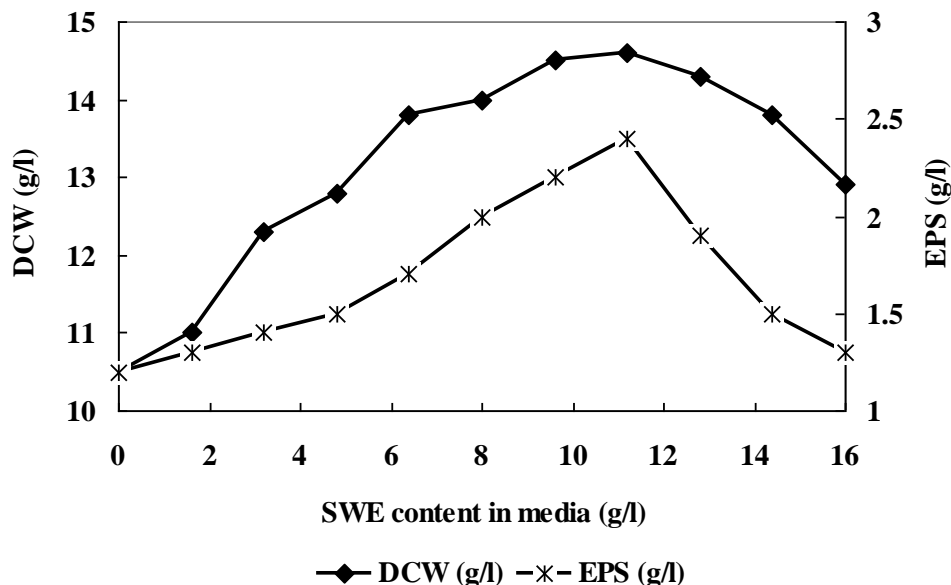
Figure 1 shows that the *S. flavescens* water extract promoted biomass growth and extracellular polysaccharides production. However, the SWE concentration should be regulated (approximately 11 g/l) such that both DCW and EPS are maximized for product production. SWE concentrations above 11 g/l favour 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.

#### *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 ( $\eta_a$ ) 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



**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.

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

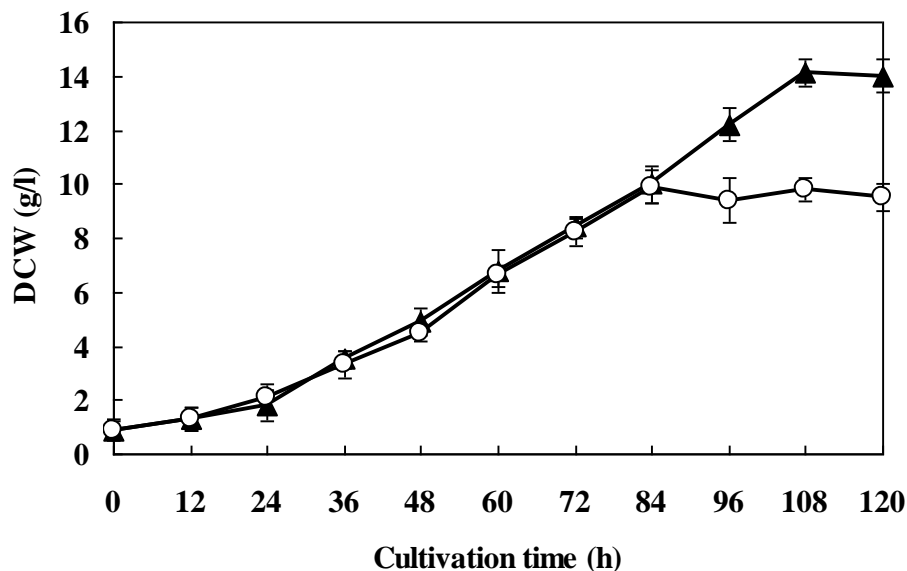
The extraction fractions obtained with n-hexane, chloroform, ethyl acetate, n-butanol and water successively were added into *G. lucidum* cultivation media separately. The control medium was only a basic medium without any *S. flavescens* extract. Figure 5 showed the DCW and EPS of the culture broths 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 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.

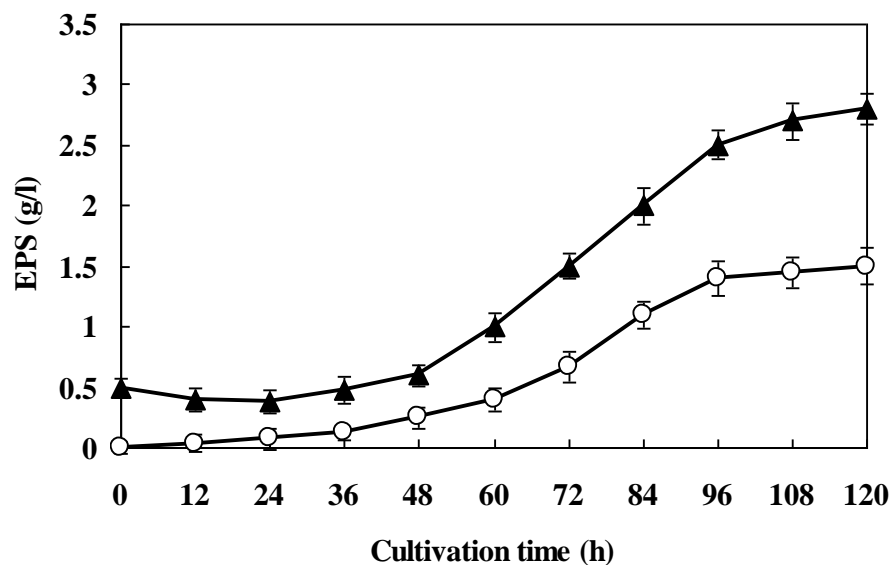
It is valuable to further investigate which compound(s) in the n-butanol extract could have the notable promoting effect on extracellular polysaccharides production.

### 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 into the 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, 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



(a)



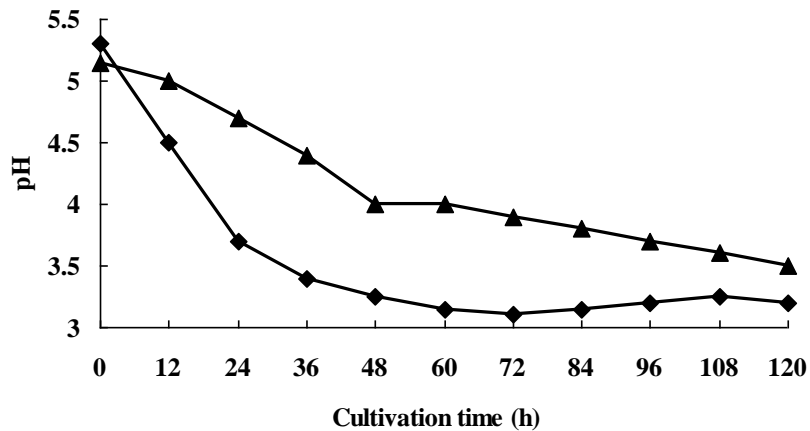
(b)

**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.

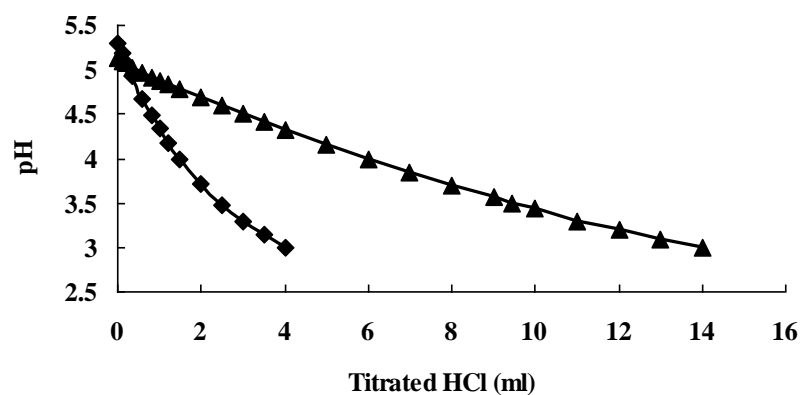
polysaccharides isolated from a fungus, *Tuber aestivum vittad* to increase biomass and extracellular polysaccharides production of *G. lucidum*. However, the mechanisms are still unknown. Moreover, the studies on the effects of herbal medicine extracts were rarely reported. In this current study, it 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

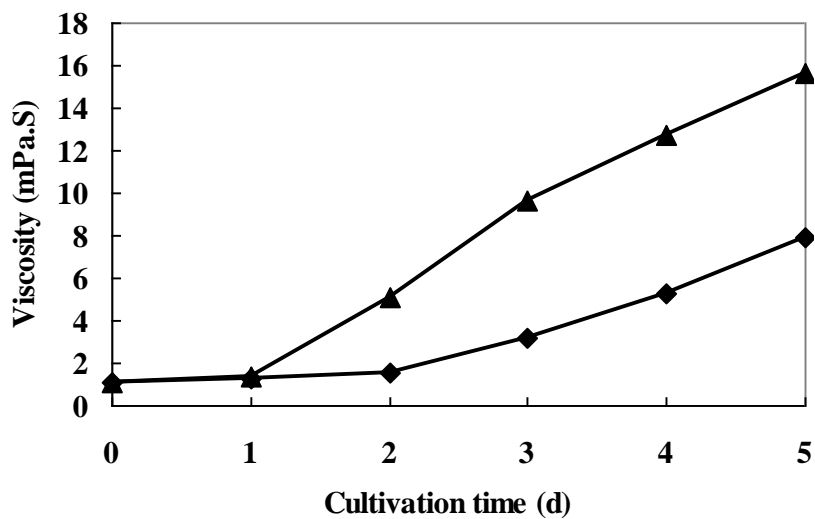


(a)

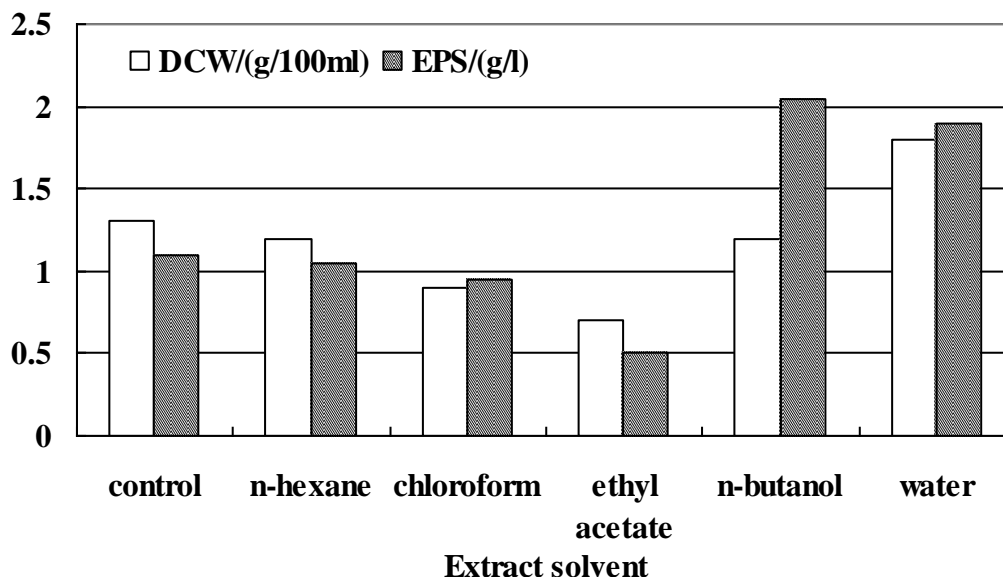


(b)

**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.



**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.

acetate extract and the promoting factors of extracellular production exist in n-butanol extract in addition 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.

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