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Improvement of crude triterpenoid and extracellular polysaccharide production by fermentation of *Lignosus rhinocerus* under the inducement of different kinds of aqueous herbal extracts

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The main purpose of this research is to improve the production of crude triterpenoid and extracellular polysaccharide of *Lignosus rhinocerus* by liquid fermentation to satisfy market demand. As we all known, the low yield of the two bioactive metabolites is a difficult problem for further research. In this paper, eleven aqueous herbal extracts were added to the fermentation of *L. rhinocerus* to increase the final concentration of the two target products.

According to the 'one-factor-at-a-time' method, Radix polygoni multiflora and Chamomile extract were identified to be effective elicitors for the improvement of mycelial biomass, cEPS and crude triterpenoid production. The peak values of mycelial biomass (i.e., 23.97 ± 0.31 g/L) and cEPS (i.e., 6.97 ± 0.56 g/L) were obtained under the concentration of 15 and 5 g/L from Radix polygoni multiflora extract, respectively. Moreover, the maximum crude triterpenoid production reached 8.72 ± 0.20 mg/g with the addition of Chamomile extract under the concentration of 25 g/L. In order to attain higher production of the total crude triterpenoid, the two optimal aqueous herbal extracts were chosen for the further research. The highest crude triterpenoid production (i.e., 20.32 ± 0.29 mg/g) and the maximum total crude triterpenoid production (i.e., 397.99 ± 5.94 mg/L) were received when the concentration of Radix polygoni multiflora and Chamomile extract was 25 g/L, which had a 3.66 and 3.05 times increase compared to the control without elicitors. This value is so far the best total crude triterpenoid production obtained in liquid fermentation of *L. rhinocerus*. This work is useful to the further study of *L. rhinocerus* for the production of polysaccharides and triterpenoid on large scale.

Keywords: *Lignosus rhinocerus* ▪ Extracellular polysaccharides ▪ Crude triterpenoid ▪ Mycelial biomass ▪ Aqueous herbal extracts

Introduction

Medicinal mushroom has long been regarded as effective medicine for the treatment of various human diseases, such as tuberculosis, asthma, coughs, and chest complaints [1,2]. Lignosus rhinocerus (Polyporaceae), a mushroom-like higher fungus, was regarded as valuable traditional medicine by local communities in Malaysia and China [3]. The sclerotium of *L. rhinocerus* is so rare and attempts have been made to cultivate this precious mushroom, in recent years, the sclerotium has become a highly prized local medicine. It is used by Chinese physicians in Hong Kong to treat liver cancer, gastric ulcers, and chronic hepatitis [4]. Previous chemical surveys on *Lignosus rhinocerus* concentrated mainly on its proximate composition and other nutritional components, such as fatty acids, proteases, steroids, minerals, and β-glucans; in particular, the physicochemical and functional features of the sclerotial dietary fibers have been widespreadly investigated. Among the bioactive ingredients in *L. rhinocerus*, the water-soluble, protein-carbohydrate complexes and β-glucans have been thoroughly studied for anti-tumor [5] and immunomodulatory effects.

As we all know, the bioactivity of mushrooms comes from two major bioactive components, triterpenoids and polysaccharides. On the other hand, little information on the major ingredients is available even though the use of *L. rhinocerus* as folk medicine for overall wellness and cancer treatment [6] might be ascribed to the existence of polysaccharides and triterpenoids with antioxidative (reduction of oxidative stress) and/or cytotoxic effects against cancer cells. Wild-

growing *L. rhinocerus* supplement the main source of these mushrooms; however, provision is confined due to their rarity [6,7]. For this reason, many researches have been reported about the yield of sclerotium [8]. However, most of these studies were focused on the ingredients of the sclerotium due to straight emphasis on the wild sclerotium which lead to neglect of mycelia. In the fact, the sclerotium is a compact mass of hardened mycelium. Very few reports addressed the bioactivities of mycelia and culture broth from liquid fermentation, both of which might be alternatives to the sclerotium. Because of this, Lau et al. [5] reported that the aqueous methanol extracts of mycelia and culture broth from shaken or static conditions showed higher or comparable antioxidant capacities to that of aqueous methanol extract prepared from the sclerotium.

Now that we need too much concentration to study the bioactive components, one of the major obstacles is the low yield of metabolites. Although polysaccharides and triterpenoid production could be improved by process optimization, the yield of the target metabolites is still too low to meet the high market demand. In plant cell culture, many methods had been used to solve the problems, and among which, elicitor was seemed as an effective one to improve the production of bioactive metabolites such as polysaccharides and triterpenoid [2]. Elicitors are chemicals or biological factors from a variety of sources that can lead physiological changes of the targeted living organism. Elicitor inducing the biosynthesis of secondary metabolites has been received wide acceptance because of its ability to significantly improve the cell culture system productivity. Induction using an elicitor is an effective method of enhancing the production of secondary metabolites such as ginseng saponin [9]. For the elicitation of secondary metabolite biosynthesis, heavy metal ion is a kind of widely used elicitor. It has been applied to induce biomass, the accumulation of terpenoid, or other secondary metabolites [10-13]. Zhu et al. [2] reported the enhanced production of ganoderic acids under induction by a microbial polysaccharide in the submerged culture of *G. lucidum*. Besides the improvement of metabolites accumulation, there is also some researches aim at the accumulation of biomass. Such as demonstrated in the culture of edible mushroom Pleurotus sajorcaju, the biomass was increased by 15-26% by adding plant growth hormones [14]. However, more researches showed that the cell growth was inhibited or not influenced while there was a markedly stimulative effect on the metabolites production.

In this study, in order to further improve the production of bioactive metabolites, the mycelia production requires to be enhanced for more research. This research focused on exploring the addition of different kinds of aqueous herbal extracts to the medium as a means of enhancing the production of mycelial biomass and bioactive compounds of *L. rhinocerus* in fermentation culture. To our knowledge, this is the first time that aqueous herbal extracts were used as the elicitor to enhance mycelial growth and production of metabolites for *L. rhinoceros*.

Materials and methods

Chemicals

Methanol and acetonitrile were of HPLC grade, and the other chemicals were of analytical reagent grade. The working mobile phase solutions were passed through a 0.22 μm membrane filter before used. They were prepared daily as needed.

Microorganism and culture conditions

The strain of *Lignosus rhinocerus* was purchased from the Sanming Mycological Institute in Fujian Province of China, and was maintained on Potato Dextrose Agar (PDA) slants, stored at 4°C. The mycelia of slants were transferred to PDA plates and incubated at 27°C for 8 days. Without otherwise stated, all experiments were performed with three replications.

L. rhinocerus was taken from the plate and grown in a 250 mL flask containing 75 mL seed culture medium at 25°C for 4 days with shaking at 180 r/min. A 7.5 mL portion of the seed culture was inoculated at 10% (v/v) into a 250 mL flask containing 67.5 mL the fermentation culture at 25°C for 4 days with shaking at 180 r/min. The seed culture medium contained (g/L): glucose 40, cornmeal 20, bran 10, KH_2PO_4 2.25, $MgSO_4$ 1.5. The fermentation culture contained (g/L): sucrose 50, yeast extract 5, KH_2PO_4 1.5.

Aqueous herbal extracts preparation

Eleven aqueous herbal extracts (Chamomile, Bamboo leaf, Pueraria, *Astragalus membranaceus*, *Rhizoma chuanxiong*, *Eucommia ulmoides*, *Cortex mori*, Ginkgo leaf, White peony, Licorice and Radix polygoni multiflora) were investigated in this study. The herbals were dried and milled. They were ground to pass a 100-mesh screen. Two-gram of each dried herbal powder was then extracted using 40 mL boiled water for 30 min. twice. The liquid portions were separated by centrifugation after extraction; the aqueous

extracts were concentrated to make up a total volume of 100 mL. These aqueous extracts were considered as a 20% (w/v) crude elicitor solution (20 g/100 mL). Each water extract was added to the fermentation culture at a concentration of 5 g/L, respectively.

Determination of mycelial biomass

To determine the mycelial biomass, *L. rhinocerus* mycelia were collected by centrifuging at 10000 rpm for 5 min. The precipitate was washed twice using sterile distilled water and dried in an oven at 65°C or freeze-dried to a constant weight. Such dry mycelial biomass was gravimetrically determined. The harvested mycelia were milled and were ground to pass a 100-mesh screen. All experiments were performed in three replicates.

Measurements of crude extracellular polysaccharides

In order to measure the production of crude Extracellular Polysaccharides (cEPS), after removal of mycelia by centrifugation, the resulting supernatant was precipitated with addition of ethyl alcohol by four times of volume and left overnight at 4°C [15]. The precipitated cEPS was collected by centrifugation at 10000 rpm for 5 min. The cEPS were dissolved in 200 mL sterile distilled water at 80°C for 1 h [16], and the solution was measured by phenol–sulfuric acid method (1.6 mL 6%phenol, 7.5 mL sulfuric acid with 2 mL solution, then stand about 20 min) [17]. The analysis of cEPS was detected at 490 nm using a spectrophotometer (UV-1600) by measuring the absorbance. The content of cEPS was calculated on the basis of a standard curve prepared by using glucose. All experiments were performed in three replicates.

Assay of crude triterpenoid

The determination of the crude triterpenoid content was conducted according to the method described by Tsujikura et al. [18]. The milled mycelia powder (ca. 1000 mg) were added with 80% (v/v) ethanol by 1:40 (w/v), water bath extraction 50 min at 80°C for three times. After removal of the mycelia by centrifugation, the supernatant was dried at 40°C using a vacuum evaporator. The residues were suspended by 30 mL water, and then 30 mL ethyl acetate was added for extraction. After removal the upper water layer, the crude triterpenoid in ethyl acetate was extracted with 30 mL ethyl acetate again. Ethyl acetate was removed by evaporation at 35°C and the resulting residues were dissolved in chromatographic grade methanol as the crude triterpenoid. The analysis of crude triterpenoid was detected at 254 nm in a spectrophotometer (UV-1600) by measuring the absorbance. The content of crude triterpenoid was calculated on the basis of a standard curve prepared by using ursolic acid. All experiments were performed in three replicates.

Statistical analysis

To choose the appropriate herbs from eleven aqueous herbal extracts for further research, the experimental design consisted of eleven variables (including the eleven herbs as above) and their interactions with three replications per treatment. The experimental data were evaluated in the SPSS 20.0 and significant effects ($p<0.05$) were recorded. Duncan's Multiple Range Test (DMRT) was performed for comparison of variables pair-wise and the significantly different effects were represented by different alphabets.

Results and discussion

Effects of different kinds of aqueous herbal extracts on mycelia growth and production of metabolites.

As described by introduction, herbs were well-known to be rich in various bioactive components; they can enhance mycelia growth and metabolite production [19,20]. Eleven aqueous herbal extracts were added to the media at quantity of 5 g/L. Measurements of mycelial biomass and metabolite production were carried in three replicates.

FIGURE 1 clearly indicates that the seven kinds of aqueous herbal extract from Chamomile, Bamboo leaf, Pueraria, *Astragalus membranaceus*, *Rhizoma chuanxiong*, *Eucommia ulmoides* and *Cortex mori* inhibited the mycelial biomass of *Lignosus rhinoceros*. The mycelial biomass was slightly enhanced by the aqueous herbal extracts from Ginkgo leaf, White peony and Licorice, while the extract of Radix polygoni multiflora was the most effective. Compared with the control, the biomass with the addition of Radix polygoni multiflora extract increased from 19.70 to 25.80 g/L, which was increased by 31.6%. This might be due to the fact that the permeability of cell wall is increased by chemical constituents in herbs, which is beneficial for uptake of nutrients.

As shown in **FIGURE 2**, it's interesting to find that apart from the extracts of Radix polygoni multiflora and Ginkgo leaf, the other extracts of herbs seemed to have a reverse effect on the crude Extracellular Polysaccharides (cEPS). Compared with the control, cEPS production was enhanced by 17.4% under the addition of Ginkgo leaf extract. On the other hand, the elicitation of

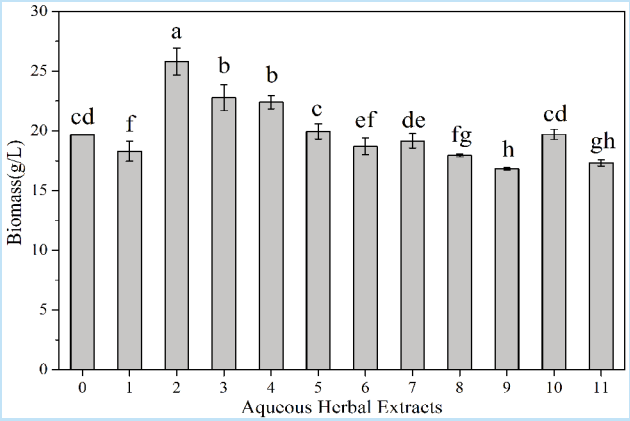

Figure 1. Effects of different kinds of aqueous herbal extracts on biomass. The different herbs were evaluated as 0: Control; 1: Chamomile; 2: *Radix Polygoni multiflori*; 3: Ginkgo Leaf; 4: White Peony; 5: Licorice; 6: Bamboo Leaf; 7: Pueraria; 8: *Astragalus membranaceus*; 9: *Rhizoma chuan Xiong*; 10: *Eucommia ulmoides*; 11: Cortex Mori. Data are the means of three independent samples, and vertical bars show standard errors. Different alphabets indicate significant differences between the lines (P<0.05, according to DMRT).

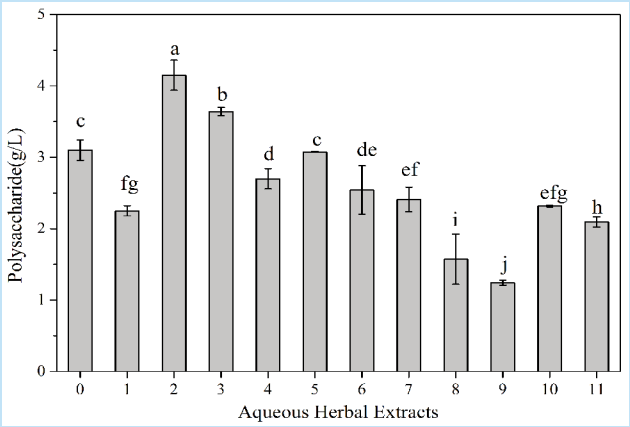

Figure 2: Effects of different kinds of aqueous herbal extracts on polysaccharide. The different herbs were evaluated as 0: Control; 1: Chamomile; 2: *Radix Polygoni multiflori*; 3: Ginkgo Leaf; 4: White Peony; 5: Licorice; 6: Bamboo Leaf; 7: Pueraria; 8: *Astragalus membranaceus*; 9: *Rhizoma chuan Xiong*; 10: *Eucommia ulmoides*; 11: Cortex Mori. Data are the means of three independent samples, and vertical bars show standard errors. Different alphabets indicate significant differences between the lines (P<0.05, according to DMRT).

Radix polygoni multiflora extract resulted in a 33.9% increase of cEPS production, which was the maximal content of 4.15 g/L.

Crude triterpenoid is one of the secondary metabolites in the fermentation of *L. rhinoceros* though the metabolic pathway of crude triterpenoid production in this fungus is not clear so far. With respect to the content of the crude triterpenoid, **FIGURE 3** shows that aqueous herbal extracts of Bamboo leaf, Pueraria, *Astragalus membranaceus*, *Rhizoma chuanxiong*, *Eucommia ulmoides*, *Cortex mori*, White peony, Licorice and Radix polygoni multiflora had a negative effect. The content of crude triterpenoid showed a slight change compared to the control when Ginkgo leaf extract was added, which improved the crude triterpenoid production by 21.6%. At the same time, the elicitation of Chamomile extract resulted in higher crude triterpenoid production than Ginkgo leaf extract, which was enhanced from 5.20 mg/g of the control to 6.86 mg/g.

To conclude, the highest mycelial biomass (i.e., 25.80 ± 1.13 g/L) and the production of cEPS (i.e., 4.15 ± 0.21 g/L) were obtained under the elicitation of Radix polygoni multiflora extract. The maximal content of crude triterpenoid (i.e., 6.86 ± 0.03 mg/g) was obtained with the addition of Chamomile extract. They were enhanced by 31.6, 33.9 and 31.8% compared with the control without addition of aqueous herbal extracts, respectively.

Effects of different concentrations of aqueous herbal extracts on mycelia growth and production of metabolites

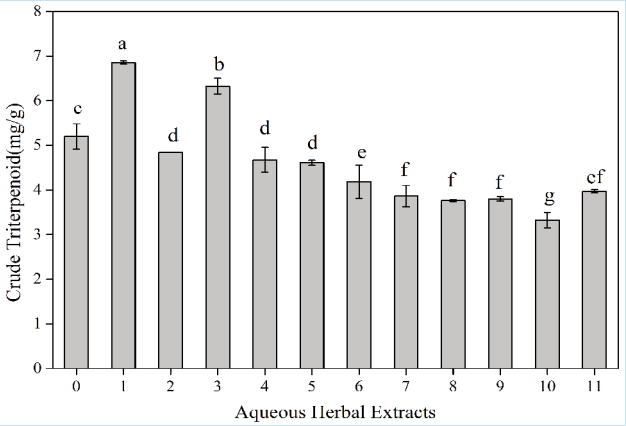

Figure 3. Effects of different kinds of aqueous herbal extracts on crude triterpenoid. The different herbs were evaluated as 0: Control; 1: Chamomile; 2: *Radix Polygoni multiflori*; 3: Ginkgo Leaf; 4: White Peony; 5: Licorice; 6: Bamboo Leaf; 7: Pueraria; 8: *Astragalus membranaceus*; 9: *Rhizoma chuan Xiong*; 10: *Eucommia ulmoides*; 11: Cortex Mori. Data are the means of three independent samples, and vertical bars show standard errors. Different alphabets indicate significant differences between the lines ($P<0.05$, according to DMRT).

The earlier results indicated that Chamomile and Radix polygoni multiflora were the optimal herbs for L. rhinoceros fermentation. The dosage of an elicitor is a main factor affecting cell growth and metabolite yields for a specific culture system [21]. Thus, experiments for aqueous herbal extracts concentration were carried out first. Too much aqueous herbal extract might inhibit the cells at a very early stage and too little aqueous herbal extract might have very little excitative effects to enhance mycelial biomass and metabolite yields. For this reason, various amounts of aqueous herbal extracts were added to the culture broth to study the effect of concentration and also to determine a suitable addition level. Measurements of mycelial biomass and metabolite yields were carried in three replicates.

As shown in **FIGURE 2a**, with respect to the influence of different-level additions on the formation of crude triterpenoid, no matter how much aqueous herbal extract of Chamomile was added, the content of the crude triterpenoid in the mycelia became more, compared with the control. However, there was no stimulative effect on the mycelial biomass. The conditions beneficial to the crude triterpenoid could have a negative effect on the mycelia growth, which was consistent with the published paper [21]. As indicated in **FIGURE 2a**, the crude triterpenoid content increased significantly at the addition quantity of 5 and 25 g/L Chamomile extract. When the amount of aqueous herbal extract of Chamomile added with the level of 5 g/L, the content of crude triterpenoid in the mycelia was enhanced from 5.65 mg/g of the control to 7.20 mg/g, which improved the crude triterpenoid production by 27.4%. Similar results were found under the concentration of 25 g/L, the content rose from 5.65 mg/g of the control to 8.72 mg/g, which improved the crude triterpenoid production by 54.3%. As previous describe, too much aqueous herbal extract might inhibit the cells at a very early stage. On this account, the concentration of Chamomile extract was controlled within 25 g/L.

Based on the results described in **FIGURE 2b**, it was obvious that without the addition of Radix polygoni multiflora extract, mycelial biomass production was low. By comparing with the control, mycelial biomass content was enhanced under the elicitation of different concentrations of Radix polygoni multiflora extract, while crude triterpenoid contents and cEPS had no evident changes under the elicitation. The elicitor might cause the lengthening of mycelia growth and also lead to the growth of the mycelia in different morphologies, which reached a higher biomass concentration and lower content of crude triterpenoid [14]. It is interesting to notice that the addition level of lower or higher than 15 g/L seemed to be advantageous to mycelia growth. Mycelial biomass production came to the maximum of 23.97 g/L on the concentration of 15 g/L, which had a 1.42 times increase. For the cEPS, the maximum production of 6.97 g/L is obtained for a concentration of 5 g/L in the fermentation. It is assumed that Radix polygoni multiflora extract might have something to do with the cEPS. These results show that the concentration of Radix polygoni multiflora extract for maximum cEPS production was different from that needed for mycelial biomass production **(FIGURE 4)**.

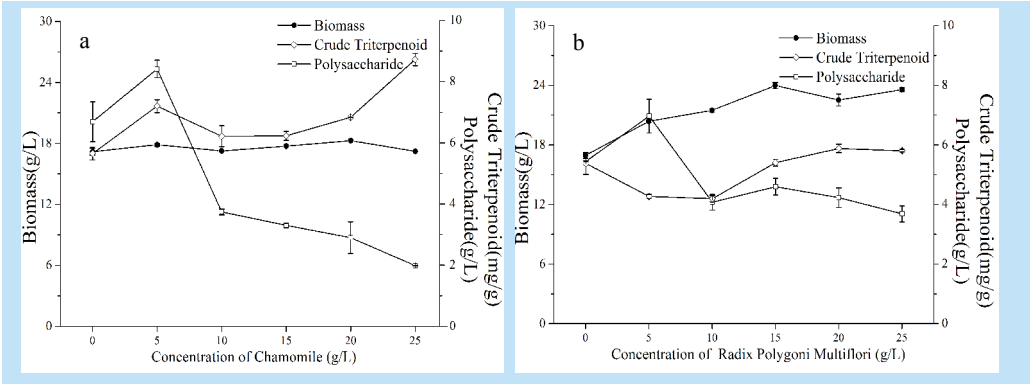

Figure 4. Effects of different concentrations of aqueous herbal extracts on mycelia growth and production of metabolites. Data are the means of three independent samples, and vertical bars show standard errors.

In conclusion, the highest mycelial biomass (i.e., 23.97 ± 0.31 g/L) and the maximal production of cEPS (i.e., 6.97 ± 0.56 g/L) were obtained under the concentration of 15 g/L and 5 g/L from Radix polygoni multiflora extract, respectively. The maximum content of crude triterpenoid (i.e., 8.72 ± 0.20 mg/g) was obtained with the addition of Chamomile extract under the concentration of 25 g/L. With the optimal concentrations of aqueous herbal extracts resulted in the maximal content we obtained, these values were used for references in the rest part of this research.

Combined effect of different concentrations of Radix polygoni multiflora extract and Chamomile extract on mycelia growth and production of metabolites

The earlier results indicated that Radix polygoni multiflora extract and Chamomile extract were the optimal aqueous herbal extracts for *L. rhinoceros* fermentation. Elicitor's addition concentration was also proved to be effective for the products. It is essential to optimize the addition concentration of the two aqueous herbal extracts by using the statistical approach of Full Factorial Designs (FFD), which could be helpful to identify and quantify their combined effect. As shown in the **TABLE 1**, the ranges for FFD experiments were selected based on the previous studies of 'one-factor-at-a-time' [22]. The design matrix of the variables in coded units and the experimental results by tests planned according the 32 FFD were presented in **TABLE 2**.

For the mycelial biomass, as shown in **TABLE 2**, the difference of every value was small. With the addition of aqueous herbal extracts, the mycelial biomass was even lower than that obtained without the addition of aqueous herbal extracts.

Table 1. Experimental range and levels of the independent variables.

Level	Independent Variables	
	X1: Concentration of Radix polygoni multiflora extract (g/L)	X2: Concentration of Chamomile extract (g/L)
1	15	5
2	20	15
3	25	25

Table 2. 3^2 full factorial design matrix and the results.

Run	X1 (g/L)	X2 (g/L)	Results			
			Mycelial Biomass (g/L)	cEPS Production (g/L)	Crude Triterpenoid Production (mg/g)	Total Crude Triterpenoid Production (mg/L)
1	15	5	19.12 ± 0.17	4.41 ± 0.25	8.62 ± 0.01	164.70 ± 0.92
2	15	15	19.02 ± 0.05	5.32 ± 0.00	12.19 ± 0.42	231.95 ± 1.94
3	15	25	17.83 ± 0.14	2.38 ± 0.04	13.29 ± 0.04	236.93 ± 5.90
4	20	5	22.33 ± 0.08	3.78 ± 0.19	8.16 ± 0.05	182.18 ± 4.69
5	20	15	19.50 ± 0.37	4.71 ± 0.37	12.19 ± 0.18	237.61 ± 8.03
6	20	25	18.32 ± 0.15	2.27 ± 0.01	14.29 ± 0.72	261.82 ± 5.85
7	25	5	20.81 ± 0.17	4.19 ± 0.20	8.86 ± 0.03	184.50 ± 11.12
8	25	15	18.72 ± 0.27	2.97 ± 0.15	9.83 ± 0.00	183.97 ± 3.51
9	25	25	19.59 ± 0.11	2.08 ± 0.02	20.32 ± 0.29	397.99 ± 5.94
0	0	0	23.50 ± 0.59	4.04 ± 1.27	5.55 ± 0.53	130.36 ± 12.72

This indicated that the mixture of aqueous herbal extracts showed a little negative effect on the cell growth. Fortunately, this little negative effect didn't lead to a negative result.

For the cEPS, in the presence of a low level of Radix polygoni multiflora extract (15 g/L), the production was increased from 4.41 to 5.32 g/L with increasing concentration of Chamomile extract levels from 5 to 15 g/L. Further increasing the concentration of Radix polygoni multiflora extract from 20 to 25 g/L and Chamomile extract resulted in no further increase even decrease in the cEPS production. Therefore, the combination of the two herbs didn't be selected for accumulation of cEPS.

For the crude triterpenoid, in the presence of concentration of Radix polygoni multiflora extract at 15 g/L, the production was increased from 8.62 to 13.29 mg/g with increasing concentration of Chamomile extract levels from 5 to 25 g/L. Further increasing the concentration of Radix polygoni multiflora extract from 20 to 25 g/L resulted in the same trend. The highest crude triterpenoid production (i.e., 20.32 ± 0.29 mg/g) was found at a Radix polygoni multiflora and Chamomile extract concentration of 25 g/L. Compared with the control, the results display the crude triterpenoid production had a 3.66 times increase. Just as above-mentioned example, the conditions favorable to the crude triterpenoid may have a negative effect on the mycelia growth, which was consistent with the paper published by Shih [23].

For the total crude triterpenoid production, it was the most important target for *L. rhinoceros* fermentation and can be obtained by Mycelial biomass × Crude Triterpenoid Production. As the results shown in **TABLE 2**, with respect to the influence of different-combination additions on the total crude triterpenoid, no matter how much extract was added to the media, the content of total crude triterpenoid in mycelia was enhanced compared with the control. When the addition of a high level of Radix polygoni multiflora extract (20 g/L) and Chamomile extract (25 g/L), the contents of total crude triterpenoid in mycelia was enhanced from 130.36 to 261.82 mg/L, which had more than twofold increase. Similar results were found in the other combination, when the addition of a high level of Radix polygoni multiflora extract (25 g/L) and Chamomile extract (25 g/L), the contents of total crude triterpenoid in mycelia was reached the maximum production that enhanced from 130.36 ± 12.72 mg/L to 397.99 ± 5.94 mg/L, which had more than threefold increase.

Conclusion

Since *Lignosus rhinoceros* is the most valuable medicinal mushroom and the wild-growing sclerotium of *L. rhinoceros* has high medicinal value, so it's precious in Hong Kong and Malaysia. As everyone knows, the triterpenoids and polysaccharides are two major active ingredients. It's very meaningful to enhance the production of them, eleven kinds of aqueous herbal extracts elicitors were investigated in this paper. For mycelial biomass and cEPS production, the results indicated that the extract of Radix polygoni multiflora was the most effective. With the addition of Radix polygoni multiflora extract under the concentration of 15 and 5 g/L, the biomass and cEPS production reached maximum value of 23.97 ± 0.31 g/L and 6.97 ± 0.56 g/L, respectively. For the total crude triterpenoid production, when the combined addition of a high level of Radix polygoni multiflora extract (25 g/L) and Chamomile extract (25 g/L), the contents of total crude triterpenoid came to the maximum of 397.99 ± 5.94 mg/L, which enhanced by 205% compared with the control. This showed the strategy of combined extracts addition was successful to satisfy both the demand of mycelial biomass and the crude triterpenoid to result in higher total crude triterpenoid production. This is the first report about the effect of aqueous herbal extracts on the bioactive metabolites accumulation in mushroom fermentation for *L. rhinoceros*.

Further studies are needed to explain the combined effect of the two herbs on the crude triterpenoid. This work also proposes an efficient approach for the development of similar strategy to enhance the other culture process for the commercial bioactive ingredients production.

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