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Optimization of Antioxidant Activities and Intracellular Polysaccharide Contents Using *Agaricus bispo*rus Extract as Elicitor in Submerged Fermenting *Ganoderma lucidum*

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Abstract

Background and Objective: *Ganoderma lucidum* is one of the medicinal fungi frequently used as supplement. The intracellular polysaccharides of this fungus include high molecular weights and help strengthen the immune system. Furthermore, these polysaccharides act as antioxidants by inhibiting free radicals and enhancing activity of the enzymes. Addition of various elicitors to the fungi submerged culture media affects the cell growth and metabolite production. Fungal extracts are one of these elicitors.

Material and Methods: In this study, *Ganoderma lucidum* was first cultured in various culture media to investigate the base media. Using three various methods (soaking the fruit body in water, disintegrating the fruit body with a blender and boiling the fruit body), *Agaricus bisporus* fruit body extract was prepared as elicitor and the extract with the highest sugar content was used. For the optimization of growth and antioxidant activity of the intracellular polysaccharides, effects of six independent factors were investigated using Placket-Burman method, including *Agaricus bisporus* extract, peptone, maltose, pH, vitamin B_1 and $CaCl_2$. Response surface method was used to optimize three factors of vitamin B_1, *Agaricus bisporus* extract and maltose. Then, stirred tank bioreactor was used to culture *Ganoderma lucidum*.

Results and Conclusion: The YPG culture medium was selected as the base medium based on mycelial growth and antioxidant activity of the intracellular polysaccharides (IC_{50}). Sugar content of the *Agaricus bisporus* extract was 30.66 µg.ml^{-1}. Placket-Burman method revealed that the extracts of *Agaricus bisporus*, maltose and vitamin B_1 significantly increased antioxidant activity of the intracellular polysaccharides. After optimizing these factors using RSM, the IC_{50} was reported as 1.047 mg.ml^{-1}. *Ganoderma lucidum* cultivation in bioreactor significantly increased the cell growth (5.29 g.l^{-1}). Intracellular polysaccharides included an IC_{50} of 1.14 mg.ml^{-1}, which was significantly higher than that the intracellular polysaccharides included in YPG culture media.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

For more than two decades, functional foods have been considered for their potential to promote health and prevent diseases [1,2]. Fungi are well-known sources of functional foods for humans. Fungi have been used in Asian countries for thousands of years due to their immune-stimulating, anti-diabetic and anti-cancer characteristics [3,4]. The medicinal *Ganoderma* (*G.*) *lucidum* fungus is a critical component of the traditional Chinese medicine. This fungus contains high concentrations of pharmacological compounds and is widely used as a dietary and medicinal supplement in the modern era. Bioactive compounds in *G. lucidum* include triterpenes, polysaccharides, proteins and

flavonoids. Over 200 polysaccharides have been isolated from this fungus, including glucose, mannose, galactose, arabinose and xylose monosaccharides [5,6]. Studies have shown that *G. lucidum* health benefits are linked to its antioxidant activity and ability to decrease oxidative damages. The ability to neutralize free radicals is likely associated to increased antioxidant enzyme activity. Researchers believe that metabolites of this fungus increase superoxide dismutase and glutathione peroxidase mRNA expression [7,8]. Numerous studies have demonstrated that compositions of the culture media can affect the fungi growth, metabolite production and biological activity [4,9]. Alvandi et al. showed that optimization of the culture media for the *Fomes fomentarius* growth significantly enhanced antioxidant and antibacterial activities of its polysaccharides [4]. According to Heydarian et al., the optimal growth and metabolite production conditions for *G. lucidum* include 35 $g.l^{-1}$ glucose in presence of vitamin B_1 and KH_2PO_4 [10]. Peng et al. reported that polysaccharide composition of *G. lucidum* changed and its antitumor activity increased significantly by increasing temperature and decreasing pH in the fungi culture media. Moreover, high levels of galactose and mannose enhanced the polysaccharide biological activity [11]. Polysaccharides of this fungus can scavenge up to 89 and 69% of DPPH and ABTS free radicals, respectively [12]. Chemical and biological elicitors with effects on defense signaling pathways have been shown to increase secondary metabolite production in fungi. Nojoki et al. reported that physical elicitors such as ultrasound increased production of the *G. lucidum* metabolites by 34% [13]. Furthermore, effects of adding L-phenylalanine on the production of *G. lucidum* polysaccharides were investigated. Addition of 0.4 $g.l^{-1}$ L-phenylalanine resulted in 62.5% increases in the polysaccharide production. Genomic and transcriptomic analyses revealed that addition of this amino acids increased the fungal enzyme production [14].

In previous studies, effects of chemical elicitors such as rifampin, methyl jasmonate and aspirin on production of ganoderic acid by *G. lucidum* were investigated. Addition of chemical elicitors significantly increased ganoderic acid production by affecting expression of genes involved in ganoderic acid production, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) and squalene synthase (SQS) [15,16]. Several studies have shown that addition of bioelicitors to submerged cultures of fungi increases growth and production of their metabolites. One of the most important bioelicitors is fungal elicitor [17-19]. Several studies have investigated effects of these elicitors on the production of ganoderic acid and exopolysaccharides [18]. However, intracellular polysaccharides (IPS) include biological characteristics as well. High molecular weight IPS can improve macrophage function and stimulate production of antibodies. Therefore, these polysaccharides are appropriate for the production of pharmaceutical supplements [20-22]. Multiple studies have been carried out on the effects of elicitors on metabolite production and biological activity of the *G. lucidum* IPS. In the current study, effects of culture media compounds on *G. lucidum* growth and IPS antioxidant activity were investigated using Placket-Burman method and RSM (response surface method). Additionally, effects of adding *Agaricus* (*A.*) *bisporus* extract, as elicitor, on antioxidant activity of the *G. lucidum* IPS were investigated. In this study, the fungal growth and antioxidant activity were assessed using stirred tank bioreactor.

2. Materials and Methods

2.1. Materials and chemials

Casein was purchased from Quelab, Montreal, Canada. Muller-Hinton broth (MHB) was purchased from Liofilchem, Roseto Degli Abruzzi, Italy. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was provided by Sigma-Aldrich, Burlington, USA. Other chemicals were purchased from Merck, Darmstadt, Germany.

2.2. Media and culture conditions of *Ganoderma lucidum*

The *G. lucidum* was provided by the Department of Horticulture, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. Fungus was cultured on potato dextrose agar (PDA) culture media and incubated at 30 °C for seven days. To select the best culture medium for the growth and metabolite production, a colony with 5-mm^2 volume was sterile transferred from the potato dextrose agar media to an Erlenmeyer flask containing the culture media (Table 1) and incubated at 30 °C with an agitation rate of 120 rpm. After 14 days, the fungal mycelia were separated from the supernatant using centrifuge (AWEL MF 20-R, France) at 5000 g for 15 min. Then, IPS were extracted. Briefly, 1 M of NaOH was added to 100 mg of the dried mycelia, mixed well and incubated at 60 °C. After 1 h, mixture was centrifuged at 11000 g for 20 min and the resulting residue was separated, dried using freeze-dryer (Operon, Korea) and stored at -18 °C until use [23].

2.3. Investigation of the effects of elicitor addition

Previous studies have shown that elicitors increase growth and metabolite production of the fungi [15,16]. Thus, effects of $CaCl_2$, vitamin B_1 and *Agaricus bisporus* extract on the antioxidant activity of *G. lucidum* IPS were investigated. The following three methods were used to prepare 500 $g.l^{-1}$ extract from the *A. bisporus* fruit body:

.Table 1. Composition of the culture media for *Ganoderma lucidum*

Culture media	Composition ($g.l^{-1}$)
MMN (Melin Norkrans Culture Medium)	NaCl 0.025, Malt extract 5, Casein 1, $[NH_4]_2HPO_4$ 0.25, KH_2PO_4 0.5, $FeCl_3$ 0.005, CaCl2 0.05, MgSO4.7H2O 0.15, Thiamin 0.1, Glucose 10
PGC (potato extract, glucose,casein)	Potato 250, Glucose 15, Casein 1
BM (Basal Medium)	Glucose 5, Casein 1, Yeast Extract 1, KH_2PO_4 0.1
YPG (Yeast extract Pepton Glucose)	Yeast Extract 10, Pepton 5, Glucose 10
PDC (Potato Dextrose Casein)	Potato Dextrose(synthetic) 24, Casein 1
GMC (Glucose Malt extract Casein)	Glucose 10, Malt extract 5, potato dextrose 24, casein 1

1) Overall, 500 g of the mushroom fruit body (purchased form Mahdizadeh Co., Iran) were divided into small pieces in 1 l of water at room temperature (100× rpm). After 24 h, the mixture was filtered and autoclaved. 2) Generally, 500 g of the mushroom fruit body were added to 1 l of water and completely mixed using blender. The filtered mixtured was autoclaved. 3) A similar concentration was prepared and boiled for 15 min; then, the filtered liquid was autoclaved.

From each of the extracts, a 20% v v^{-1} concentration was added to YPG media on 7 Day of the fungal growth. After 14 days, mycelia and IPS were achieved and the antioxidant activity was investigated [18].

2.4. Assessment of the sugar content in *Agaricus bisporus*

In this method, monosaccharides and oligosaccharides and their derivatives with phenol and sulfuric acid produce light brown color. Glucose was used as standard for the experiment. Briefly, 50 µl of the sample and the glucose standard were added to a 96-well microplate; hence, concentration of each well was 360 ppm. Then, 150 µl of sulfuric acid were added to each well. Immediately, 30 µl of 5% aqueous phenol solution were added to each well. The plate was incubated at 90 °C for 5 min and then cooled down at room temperature for 5 min. The UV absorption was read at 490 nm [24].

2.5. Experimental design based on Placket-Burman method

Placket-Burman method provides unbiased estimates of the major effects in the smallest possible design. The major advantage of this method is that the minimum number of observations is needed to calculate effects of a particular factor. Based on the previous studies [10,11], effects of six factors were investigated using Minitab Software v.16.2.4.4 (Minitab, USA). These factors included *A. bisporus* extract, peptone, maltose, vitamin B_1, $CaCl_2$ and pH. All culture media, except the substances listed in the table, included 2.5 $g.l^{-1}$ yeast extract, 1.5 $g.l^{-1}$ KH_2PO_4 and 1 $g.l^{-1}$ $MgSO_4.7H_2O$. Overall, 35 ml of the culture media were prepared using100-ml Erlenmeyer flakes. After autoclaving, a 5-mm^2 colony of 7-day potato dextrose agar was inoculated into the media. Cultures were set in dark at 30 °C with an agitation rate of 120 rpm. Mycelia and IPS were achieved and antioxidant characteristics were assessed using DPPH assay. For each sample, DPPH assay was carried out at various concentrations (20, 30, 40, 50, 60 and 70 µl). After drawing a line graph, IC_{50} value was calculated using the line equation. The IC_{50} from each sample was considered as a test response (Table 2).

Table 2. Data from the experiments designed using Placket-Burman method

Test no.	pH	*Agaricus bisporus* extract ($mg.l^{-1}$)	Peptone ($g.l^{-1}$)	Maltose ($g.l^{-1}$)	Vitamin B_1 ($g.l^{-1}$)	$CaCl_2$ (mM)	R1:Mycelium dry ($g.l^{-1}$)	R2:Antioxidant IC_{50} ($mg.ml^{-1}$)
1	3.5	15	2.0	50	0.01	50	5.01	3.98
2	3.5	15	5.0	20	0.10	50	3.54	1.40
3	3.5	45	2.0	50	0.10	1	2.66	1.30
4	7.5	15	5.0	50	0.01	50	4.96	3.52
5	3.5	45	5.0	20	0.10	50	0.45	10.84
6	7.5	45	2.0	50	0.10	50	2.74	4.25
7	7.5	15	5.0	50	0.10	1	16.28	2.50
8	3.5	45	5.0	50	0.01	1	2.72	3.29
9	7.5	45	5.0	20	0.01	1	1.49	15.82
10	7.5	45	2.0	20	0.01	50	2.26	14.65
11	7.5	15	2.0	20	0.10	1	6.41	2.08
12	3.5	15	2.0	20	0.01	1	3.54	3.65
13	5.5	30	3.5	35	0.05	10	1.52	3.42
14	5.5	30	3.5	35	0.05	10	1.50	3.48
15	5.5	30	3.5	35	0.05	10	1.50	3.42
16	5.5	30	3.5	35	0.05	10	1.49	3.45

Table 3. Data from the experiments designed using RSM

Test no.	*Agaricus bisporus* extract ($mg.l^{-1}$)	Maltose ($g.l^{-1}$)	Vitamin B_1 ($g.l^{-1}$)	R1: Mycelium dry ($g.l^{-1}$)	R2: Antioxidant IC_{50} ($mg.ml^{-1}$)
1	30	5	0.005	6.33	1.12
2	30	5	0.015	6.08	3.56
3	30	35	0.005	17.24	4.25
4	30	35	0.015	14.66	8.25
5	60	5	0.005	5.37	7.85
6	60	5	0.015	5.70	1.06
7	60	35	0.005	17.86	4.65
8	60	35	0.015	24.26	3.81
9	45	20	0.005	11.52	4.05
10	45	20	0.015	10.09	1.75
11	45	5	0.010	5.46	2.58
12	45	35	0.010	13.94	2.31
13	30	20	0.010	10.49	3.69
14	45	20	0.010	11.66	4.95
15	45	20	0.010	11.50	3.05
16	45	20	0.010	11.33	3.05
17	45	20	0.010	11.62	3.05
18	45	20	0.010	11.44	3.05
19	45	20	0.010	11.36	3.05
20	45	20	0.010	11.38	3.05

2.6. Optimization of the *Ganoderma lucidum* growth and antioxidant activity using response surface method

Based on the results from Placket-Burman method, three practical factors (*A. bisporus* extract, maltose and vitamin B_1) were identified and the practical levels of each factor were calculated based on the T-value parameter and RSM were designed using Design Expert Software v.7.0.0 (Statease, USA). The RSM could study several variables at the same time. It examines the relationships between several variables and responses and reveals the optimal response. Each factor was added to each media according to the software. Culture media were prepared in 75 ml volumes and placed in an incubator at 30 °C and 120 rpm in the dark (Table 3). Then the significance of each variable and their interaction was calculated and the appropriate equation was obtained.

2.7. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Radical capacity reduction method assessed antioxidant effects of the extracts using DPPH. Briefly, a 4 $mg.ml^{-1}$ solution of each sample in methanol and 0.08 $mg.ml^{-1}$ of DPPH in methanol were prepared. The extract and DPPH solution were mixed at various ratios and incubated at 25 °C for 30 min. Then, absorbance of the sample was measured using ELISA reader (Carry 100 Bio, Australia) and antioxidant activity was calculated using the following formula:

$$\text{DPPH} \bullet \text{ scavenging effect (\%)} = \frac{(A0 - Asample)}{A0} \times 100$$

Where, A_0 was absorbance of the control measured at 517 nm and A_{sample} was absorbance of the sample measured at 517 nm [25].

2.8. *Ganoderma lucidum* cultivation in bioreactor

After verifying optimization and increasing antioxidant activity of the IPS, the optimal conditions were set in a stirred tank bioreactor (Model SabaFerm 110, Zist Farayand Sanat Saba, Iran). The 7-day liquid culture was inoculated into the media (1% v v^{-1}). The culture media included 50 mM $CaCl_2$, 0.015 $g.l^{-1}$ vitamin B_1, 5 $g.l^{-1}$ maltose, 5 $g.l^{-1}$ peptone, 60 $mg.l^{-1}$ *A. bisporus* extract, 2.5 $mg.l^{-1}$ yeast extract, 1.5 $mg.l^{-1}$ KH_2PO_4 and 1 $mg.l^{-1}$ $MgSO_4.7H_2O$ (pH 5.5). Volume of the bioreactor was 5 l (working volume 1.5-3.8 l) and the length to diameter ratio was 2.5. The bioreactor conditions included 120 rpm, 3 °C, D_o = 40% and aeration = 1.5 $l.min^{-1}$. After 10 days, sample was removed from the bioreactor due to the saturation of culture media with the mycelia and the mycelia were separated and dried. Based on the highlighted method from the previous sections, IPS was extracted from the mycelia and lyophilized for DPPH assays.

3. Results and Discussion

3.1. Selection of the appropriate culture media

The *G. lucidum* was cultured in various media for 14 days. Then, the mycelia dry weight was measured and the appropriate culture medium was selected. Because *G. lucidum* growth was significantly ($p < 0.05$) higher in YPG and GMC culture media, these two media were designated as the basic culture media (Fig. 1A). Based on the previous studies, organic nitrogen sources and C: N ratios are critical for the fungi growth [4]. Peptone increases production of the fungal growth enzymes in YPG culture media [26]. Additionally, yeast extract is an excellent source of nitrogen, which promotes the fungal growth [27]. In contrast, malt extract includes 78% maltose as energy source that can stimulate the fungal growth and metabolism in GMC culture media [28].

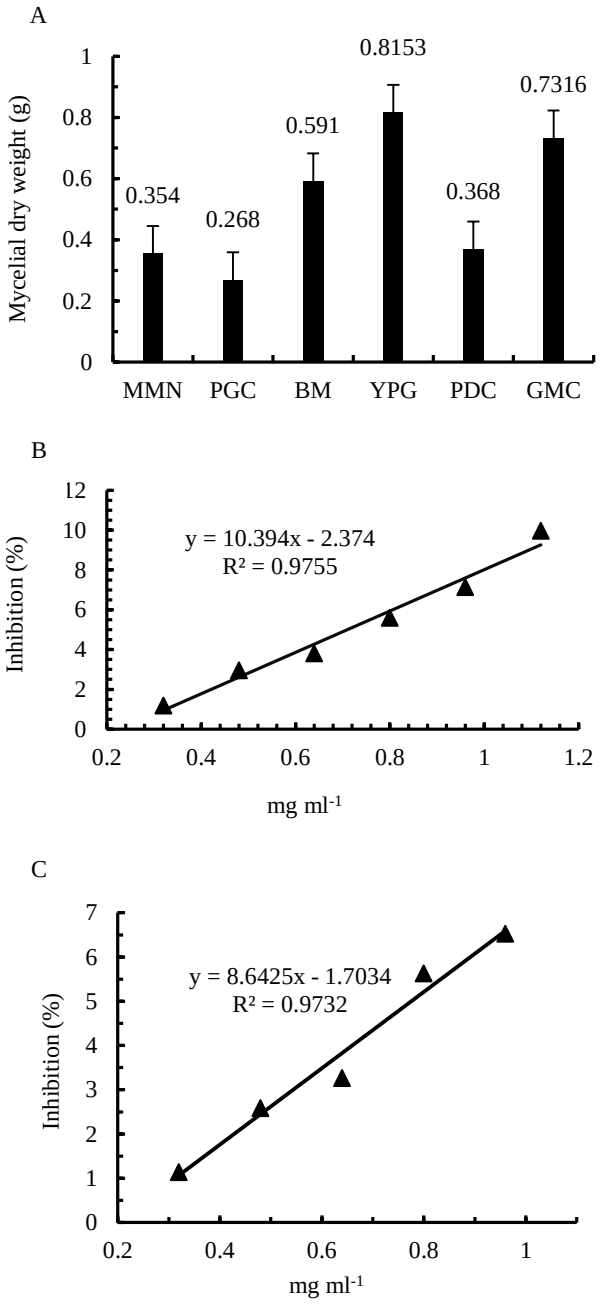


Figure 1. Comparison of dry weight of *Ganoderma lucidum* in different culture media (A). DPPH free radical's inhibition of the intracellular polysaccharides from YPG (B) and GMC (C) culture media.

Therefore, DPPH assay was used to assess the antioxidant activity of IPS from these two media (Figs. 1B and C). The IC_{50} value of IPS from YPG and GMC were 5.0388 and 5.982 mg.ml^{-1}, respectively. The antioxidant activity of *G. lucidum* polysaccharides is normally attributed to their high hydrogen contents, enabling them to form stable structures when combined with free radicals. Polysaccharides from *G. lucidum* exhibit antioxidant activity, lowering lipid peroxidation and malondialdehyde levels [29,30]. Since the IC_{50} concentration in YPG media was lower than that with a similar concentration in GMC, antioxidant activity of the IPS in this culture medium was greater than that in other media and thus this culture medium was considered appropriate for the experimental design.

3.2. Sugar content in *Agaricus bisporus*

To select the best bioelicitor with high polysaccharide content, quantities of sugar in all the three *A. bisporus* extracts were measured using phenol sulfuric acid method. Studies show that *A. bisporus* extraction methods and conditions such as temperature can affect the sugar content [31, 32]. Results of this study showed that the extract from Methods 1, 2 and 3 included 21.3, 30.66 and 24.16 µg.ml^{-1} sugar, respectively. Polysaccharides were more abundant in mushrooms that were blended. As a result, this extract was used as the culture media elicitor.

3.3. Effects of adding *Agaricus bisporus* extract to YPG media

On Day 7, addition of the *A. bisporus* extract as bioelicitor to culture media increased the fungal growth rate and antioxidant activity of its IPS. The *G. lucidum* mycelial weight in *A. bisporus* extract and YPG media reached 5.004 g.l^{-1}. Whereas, the *G. lucidum* growth decreased after seven days in YPG culture media. Due to the lack of glucose, addition of *A. bisporus* extract provided glucose for the fugal growth and metabolism, resulting in increased biomass production in the culture media. By adding the *A. bisporus* extract, a 16% increase in antioxidant activity was reported and IC_{50} of IPS included 4.25 mg.ml^{-1}. Addition of the bioelicitor could alter the polysaccharide structure, monosaccharide content and phenolic content. Based on several studies, combination of monosaccharides includes significant effects on the antioxidant activity of polysaccharides [32].

3.4. Results of Placket-Burman method

Six factors were used as experimental variables and 16 experiments were carried out. Mycelial dry weight of each culture medium was calculated. The IC_{50} of IPS for each culture medium was then considered as response to the significant factors (Table 2). While increased biomass naturally produces further significant quantities of polysaccharides, it does not always result in increased antioxidant activity. Studies have demonstrated that higher fungi include distinct growth and production patterns for metabolites and differences in composition of the culture media result in differences in biological activity of the fungal polysaccharides [4,33]. The R^2 of this experiment was 86%, which showed reliability of the results. Based on data analysis, three factors of vitamin B_1, maltose and *A. bisporus* extract significantly affected the antioxidant activity ($p < 0.05$). By providing the necessary glucose quantity for the fungal growth, maltose and *A. bisporus* extract can stimulate and preserve the fungal growth and

metabolism. Additionally, the sugar can alter structure of the polysaccharide chain and enhance their biological activity [32]. Vitamin B_1 is a cofactor in carbohydrate and amino acid metabolisms and is needed for glycolysis and the tricarboxylic acid cycle. Vitamin B_1 has been shown to increase the content of phenols and flavonoids by affecting gene expression. Increased phenolic content is directly linked to the antioxidant activity of polysaccharides [4,34,35]. Important factors for the fungal growth and antioxidant activity included 50 mM $CaCl_2$, 0.01 $g.l^{-1}$ vitamin B_1, 20 $g.l^{-1}$ maltose, 5 $g.l^{-1}$ peptone and 45 $mg.l^{-1}$ *A. bisporus* extract (pH 5.5).

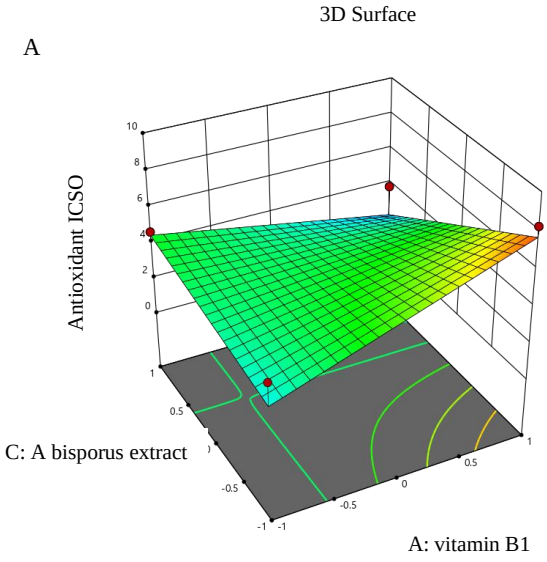
3.5. Optimization of the *Ganoderma lucidum* IPS antioxidant activity using response surface method

Based on the previous step, 20 RSM sets were carried out [4,33,36]. The R^2 of this test was 85.77% (adjusted R^2 was 77%). This value showed that the responses were reliable. This model was valid and significant ($p < 0.05$). However, the lack of fit was not significant ($p = 0.06$). Based on the *p*-value of the factors, Variables B, AB, BC and C^2 included significant effects ($p < 0.05$). The *p*-value of AC was significant ($p < 0.01$). The final equation for the antioxidant activity of IPS with the coded expressions is as follows:

Antioxidant activity = 3.00 + 0.71B + 0.94AB - 1.76AC - 1.03BC + $1.32C^2$

Where, A was vitamin B_1, B was maltose and C was the *A. bisporus* extract.

Effects of these factors on antioxidant characteristics are shown in Fig. 2 (equivalent to decreasing IC_{50}). Figure 2A shows effects of vitamin B1 and maltose on the antioxidant activity of IPS. Furthermore, Figure 2B demonstrates effects of vitamin B1 and *A. bisporus* extract on the antioxidant activity of IPS as well as effects of *A. bisporus* extract and maltose on antioxidant activity of the IPS (Fig. 2C).



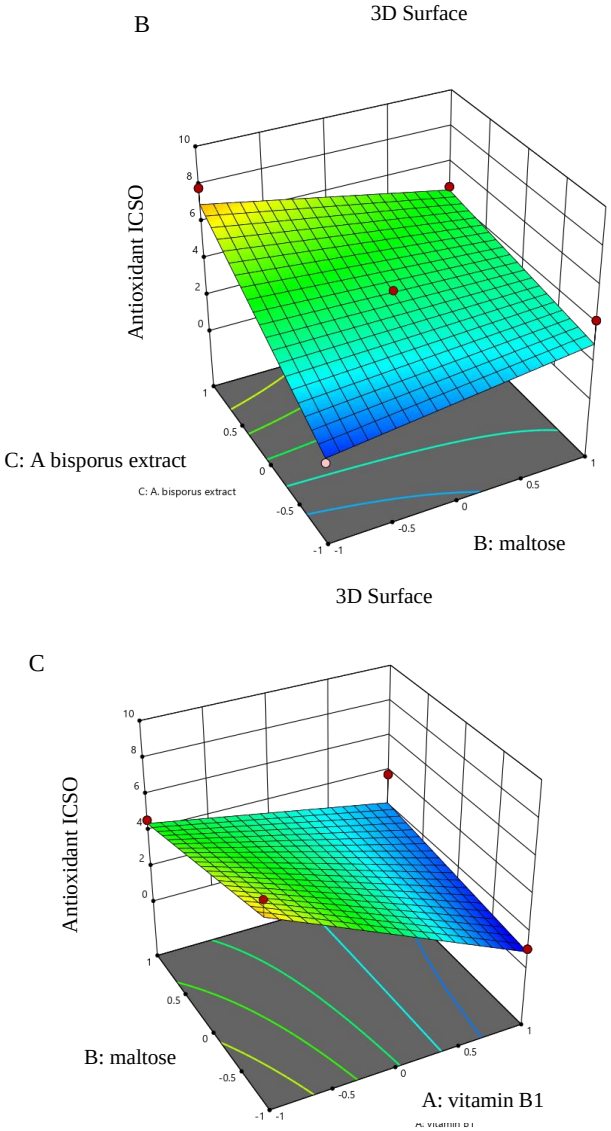


Figure 2. Effects of vitamin B_1 and maltose on antioxidant activity of the intracellular polysaccharides (A). Effects of vitamin B_1 and *Agaricus bisporus* extract on antioxidant activity of the intracellular polysaccharides (B) and effects of *Agaricus bisporus* extract and maltose on antioxidant activity of the intracellular polysaccharides (C)

Culture physicochemical conditions affected polysaccharide structure, monosaccharide composition and monosaccharide ratio. Addition of the bioelicitors to culture media increased contents of β-glucan and polyphenols. Presence of these compounds was associated to antioxidant activity and inhibition of free radicals [4,13,15]. The optimal condition included 0.015 $mg.l^{-1}$ vitamin B_1, 5 $g.l^{-1}$ maltose and 60 $mg.l^{-1}$ *A. bisporus* extract and IC_{50} decreased to 1.047 $mg.ml^{-1}$, which revealed increases in antioxidant activity. The IC_{50} predicted by the software was 1.023. Predicted and achieved values were consistent.

3.6. *Ganoderma lucidum* cultivation in bioreactor

The fungi mycelia were cultured and dried after ten days of incubation in stirred tank bioreactor. The mycelial

growth in bioreactor increased significantly ($p < 0.05$) and reached to 5.29 g l^{-1} (Fig. 3A).

The mycelial morphology in bioreactor could be filamentous or spherical and the mycelial production changed with morphology. Studies have shown that the shear stress in this bioreactor can affect production of IPS by *G. lucidum* [37,38].

Then, antioxidant activity of the fungal IPS was investigated (Fig. 3B). The IC_{50} in the optimal culture media was 1.14 mg ml^{-1}.The IC_{50} value of IPS from YPG was 5.11 mg ml^{-1}, which showed 77.7% increases in antioxidant characteristics, compared to that of IPS from the optimal culture media. Assessment of *Pycnoporus sanguineus* in stirred tank and airlift bioreactors showed that the composition of polysaccharides produced by the fungus could be different. Differences in composition and molecular weight of the polysaccharides also cause differences in antioxidant activity of the polysaccharides. Results have shown that variations in aeration and shear stress alter the mycelial morphology and hence the fungal metabolism [39,40]. Figure 3C shows *G. lucidum* cultivation in stirred tank bioreactor. Nowadays, *G. lucidum* is commercially produced and used to prevent and treat several diseases. Optimizing culture conditions of this fungus improves efficiency of the mycelial production. Numerous supplements of polysaccharides from this fungus are available in addition to its mycelia. In addition to increases in efficiency of the polysaccharide production, addition of bioelicitors and optimization of culture media increase biological activity, particularly the antioxidant characteristics.

By inhibiting free radicals and enhancing enzymatic activity, polysaccharides of this fungus can be used as dietary supplements to help prevent various diseases, especially cancers.

4. Conclusion

In conclusion, effects of using *A. bisporus* extract as bioelicitor on the growth and antioxidant activity of *G. lucidum* IPS were investigated in this study. After selecting YPG culture media as the base media, Placket-Burman method was used to assess effects of six independent factors on the fungal growth and antioxidant activity. Furthermore, RSM was used to optimize effects of the three factors including *A. bisporus* extract, maltose and vitamin B_1. The IC_{50} of the IPS was 1.047 mg ml^{-1} under optimal conditions. Assessment of the fungal growth using stirred tank bioreactor revealed increases in the fungal growth (5.29 g.l^{-1}) and IC_{50} (1.14 mg.ml^{-1}), compared to the basic culture media.

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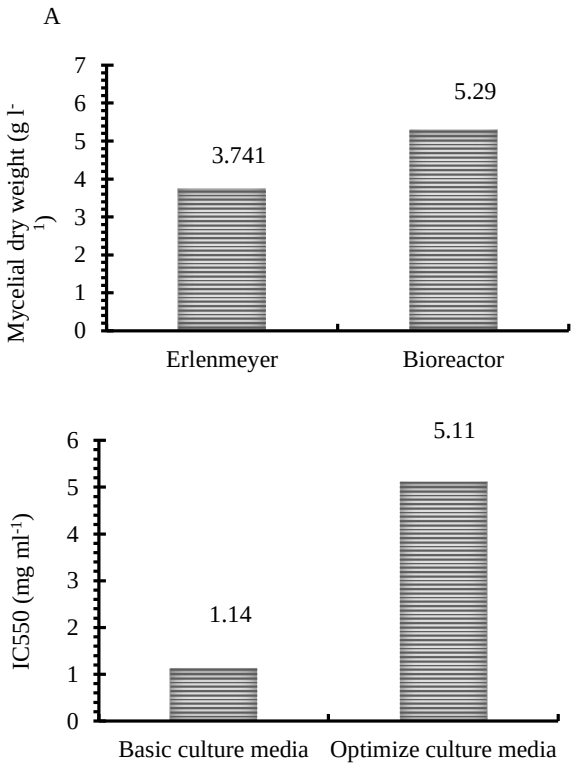

Figure 3. Comparison of *Ganoderma lucidum* mycelial dry weight in Erlenmeyer and stirred tank bioreactor (A). Antioxidant activity (IC_{50}) of the intracellular polysaccharides from *Ganoderma lucidum* culture in Erlenmeyer flasks and stirred tank bioreactors (B). Cultivation of *Ganoderma lucidum* in stirred tank bioreactor (C).

6. Conflict of Interest

The authors report no conflicts of interest.

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بهینه‌سازی فعالیت‌های ضداکسایشی و محتوای پلی‌ساکارید درون یاخته‌ای با استفاده از عصاره *آگاریکوس بیسپوروس* به‌عنوان محرک در تخمیر غوطه‌ور *گانودرما لوسیدوم*

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چکیده

سابقه و هدف: *گانودرما لوسیدوم* یکی از قارچ‌های دارویی است که بارها به‌عنوان مکمل مورد استفاده قرار گرفته است. پلی‌ساکاریدهای درون‌یاخته‌ای[۱] این قارچ وزن مولکولی بالایی دارند و به تقویت سیستم ایمنی کمک می‌کنند. علاوه بر این، این پلی‌ساکاریدها با مهار رادیکال‌های آزاد و افزایش فعالیت آنزیم‌ها، به‌عنوان ضداکسایندهٔ[۲] عمل می‌کنند. افزودن الیسیتورهای گوناگون به محیط کشت غوطه‌ور قارچ‌ها بر رشد سلولی و تولید متابولیت اثر می‌گذارد. یکی از این الیسیتورها، عصاره‌های قارچ‌ها می‌باشند.

مواد و روش ها: در این مطالعه، *گانودرما لوسیدوم* ابتدا به‌منظور انتخاب محیط کشت پایه، در محیط‌های کشت گوناگون کشت داده شد. سپس عصاره جسم میوه‌ای قارچ *آگاریکوس بیسپوروس* به عنوان الیسیتور با سه روش (خیساندن جسم میوه‌ای در آب، له کردن آن در مخلوط کن و جوشاندن آن) تهیه گردید و از عصاره با بیشترین میزان قند استفاده شد. ببهینه سازی رشد و فعالیت ضداکسایشی پلی‌ساکارید درون‌یاخته‌ای، با استفاده از روش پلاکت برمن اثرات شش متغیر مستقل شامل عصاره *آگاریکوس بیسپوروس*، پپتون، مالتوز، pH، ویتامین B_1 ، و $CaCl_2$ انجام شد. روش پاسخ سطح برای بهینه‌سازی سه عامل ویتامین B_1 ، عصاره *آگاریکوس بیسپوروس* و مالتوز مورد استفاده قرار گرفت. سپس، از بیوراکتور همزن‌دار برای کشت *گانودرما لوسیدوم* استفاده شد.

یافته‌ها و نتیجه‌گیری: با توجه به رشد میسیلیوم و فعالیت ضداکسایشی پلی‌ساکاریدهای درون‌یاخته‌ای (IC_{50}) محیط‌کشت YPG به عنوان محیط پایه انتخاب شد. محتوای قند عصاره *آگاریکوس بیسپوروس* $\mu g.ml^{-1}$ ۳۰/۶۶ بود. تجزیه و تحلیل داده‌های پلاکت برمن نشان داد، عصاره *آگاریکوس بیسپوروس*، مالتوز و ویتامین B_1 اثر معنی‌داری بر فعالیت ضداکسایشی پلی‌ساکاریدهای درون‌یاخته‌ای دارند. با بهینه‌سازی این متغیرها با روش پاسخ سطح، IC_{50} به $mg.ml^{-1}$ ۱/۰۴۷ رسید. کشت *گانودرما لوسیدوم* در بیوراکتور به طور قابل توجهی رشد ($g.l^{-1}$ ۵/۲۹) را افزایش داد. IC_{50} پلی‌ساکاریدهای درون‌یاخته‌ای به $mg.ml^{-1}$ ۱/۱۴ رسید، که در مقایسه با محیط‌کشت YPG به طور معنی‌داری افزایش داشت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] Intracellular

\ antioxidant