Journal of Bioanalysis & Biomedicine

Sharma, J Bioanal Biomed 2015, 7:6
DOI: 10.4172/1948-593X.1000141

Research Article Open Access

# Optimized Extraction and Antioxidant Activities of Polysaccharides from Two Entomogenous Fungi

**Sapan Kumar Sharma***

*Centre for Mushroom Research and Training, Department of Plant Pathology, CSK, Himachal Pradesh Agriculture University, India*

**Abstract**

Submerged cultures of two entomogenous fungi *Isaria sinclairii* and *I. tenuipes* have been optimized for polysaccharides production and their biological activities have been evaluated under present studies with using one- factor-at-a-time method. Combination of various factors influenced the higher polysaccharides production in both of the fungi. Polysaccharides production was influenced by different carbon, nitrogen and mineral sources. *In vitro* evaluation of EPS and IPS of both species showed significant antioxidant activities and high inhibition rate of peroxidation of polyunsaturated fatty acids.

**Keywords:** *Isaria sinclairii; I. tenuipes;* EPS; IPS; Antioxidants

## Introduction

Genus *Isaria* belongs to phylum Ascomycota and class Sordariomycetes with worldwide distribution [1-3]. Many Ascomycetes species have been used as the source of disease combating natural products with tremendous biological, pharmacological and immunomodulatory activities [4-10]. *Cordyceps militaris*, *C. sinensis, C. ophioglossoides* etc. have been found to contain abundant kind of active compounds and extensively used in East Asia for treatment of anti- inflammation, renal dysfunction, asthma, and cancer [11-14]. Biological active compounds extracted from many species of entomopathogenic genera have shown far reaching liver protective effects, anti-oxidative activity, enhancing the T-cell and macrophages activity, vascular endothelial growth factor levels in the lungs and liver by exopolysaccharide fraction, reduction in cholesterol and triglyceride [15-19].

Polysaccharides extracted from the mycelium of these fungi constitute the main bioactive agents and exhibit multiple pharmacological activities including antitumor, anti- inflammatory, immunopotentiation, hypoglycemic and hypocholesterolemic effects, protection of neuronal cells against the free radical- induced cellular toxicity, steroidogenesis and antioxidant activities [20-26]. High antioxidant activities provide health benefits in preventing damages due to free radicals produced by biological degeneration [27-31]. Polysaccharides protect neuronal cells against the free radical-induced cellular toxicity and stimulate steroidogenesis. Polysaccharides possess great potential and considered as important tool for studying the development of nutraceutical products. However, under submerged culture conditions, the productivity of polysaccharides has been observed to vary with environmental conditions (medium composition, carbon source, nitrogen source, pH, etc.). Commercial cultivation through submerged culture is becoming quite useful nowadays because of higher mycelial yield with fewer chances of contamination [32,33]. In view of this, present studies have been conducted to optimize the EPS and IPS production by one-factor-at-a-time method and orthogonal matrix design, and to evaluate the antioxidant activities of EPS and IPS obtained under submerged culture conditions.

## Materials and Method

### Culturing and optimization studies

*Isaria sinclairii* and *I. tenuipes* were collected on Hymenopteran and Coleopteran insect respectively from Mcleodganj, Dharamshala (Himachal Pradesh, India, 2200 m above mean sea level) and were deposited at Herbarium, Department of Botany, Punjabi University, Patiala (*I. sinclairii* PUN 7074; *I. tenuipes* PUN 7073). Isolation was done on Potato Dextrose Agar (PDA) slants by tissue culture technique (Figure 1). The slants were incubated at 25°C for 10 days. Cultures were maintained by repeated sub-culturing after 30 days. Submerged cultivation was done in a standard medium (sucrose 30.0 g/L, yeast powder 5.0 g/L, peptone 5.0 g/L, $MgSO_4 \cdot 7H_2O$ 1.0 g/L and $KH_2PO_4$ 0.5 g/L) [34,35]. Effect of medium capacity (50, 100, 150, 200 and 250 mL), rotation speed (50, 100, 125, 150, 175 and 200 rpm) of culture medium, incubation time (2-10 days), pH (3.0-8.0), temperature (20, 22, 25, 27, 30, 33°C), carbon sources (glucose, galactose, sucrose, mannitol, maltose and fructose), nitrogen sources (peptone, yeast extract, $NaNO_2$ $(NH_4)_2SO_4$ and L-Arginine HCL) and C/N (1:5, 1:10, 1:20, 1:30 and 1:40) ratio was studied by orthogonal experiments using one-factor-at-a-time method were tested for maximum EPS and IPS production.

### Extraction of EPS and IPS

EPS were extracted by the method described by Fang and Zhong

**Figure 1:** Wild fruit bodies of *Isaria sinclairii* and *I. tenuipes*.

***Corresponding author:** Sapan Kumar Sharma, Centre for Mushroom, Research and Training, Department of Plant Pathology, CSK, Himachal Pradesh Agriculture University, Palampur-176062, India, Tel: +91 9857-481-650; E-mail: sapan.84.sharma@gmail.com

**Received** April14, 2014; **Accepted** November 10, 2015; **Published** November 13, 2015

**Citation:** Sharma SK (2015) Optimized Extraction and Antioxidant Activities of Polysaccharides from Two Entomogenous Fungi. J Bioanal Biomed 7: 180-187. doi:10.4172/1948-593X.1000141

**Copyright:** © 2015 Sharma SK. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

[36] with minor modifications. Briefly, mycelial biomass in the medium was centrifuged at 10,000 × g for 12 min. The supernatant obtained was mixed with three volumes of pure ethanol and left for 24 hours at 4°C. The resulting precipitate was then separated by centrifugation at 8000 × g for 12 min. The precipitate (EPS) was washed with ultrapure water and subsequently lyophilized for quantitative assessment and analysis.

For IPS, mycelial biomass was subjected to extraction with boiling water for an hour and the mixture was filtered through Whatman no. 1 filter paper. The filtrate was allowed to precipitate using pure ethanol and left overnight at 4°C. The polysaccharides thus precipitated were separated by centrifugation at 8000 × g for 10 min. The precipitate (IPS) were washed with ultrapure water and subsequently lyophilized for quantitative analysis [37].

## Polysaccharides composition

Monosaccharide composition of polysaccharides was determined by high performance liquid chromatography coupled to an evaporative light scattering detector [38]. Polysaccharide fraction (0.1 g) was extracted with 2.5 ml of 70% aqueous methanol followed by 1.5 ml of 70% aqueous methanol and then 1 ml of 70% aqueous methanol. This extract was centrifuged at 4000 rpm at 4°C for 10 min. Supernatant was collected and volume made up to 5 ml with 70% methanol. The extract was passed through Millipore filter (0.45 µm) prior to injection on the HPLC.

## Antioxidant assays

**DPPH radical scavenging activity:** The DPPH scavenging activity was measured following standard method [39]. For this, DPPH (200 µm) solution at different concentrations (2-10 mg/mL) was added to 0.05 mL of the samples dissolved in ethanol. An equal amount of ethanol was added to the control. Ascorbic acid was used as the control. The absorbance was read after 20 min. at 517 nm and the inhibition was calculated using the formula:

DPPH scavenging effect (%)=$A_0$-$A_P$/$A_0$ × 100, where $A_0$ was the absorbance of the control and $A_P$ was the absorbance in the presence of the sample.

**ABTS radical scavenging assay:** ABTS radical scavenging activity was measured by the method described by Li et al. [40]. For this, 10 µL of the sample was added to 4 mL of the diluted $ABTS^{\bullet+}$ solution (prepared by adding 7 mM of the ABTS stock solution to 2.45 mM potassium persulfate, kept in the dark, at room temperature, for 12-16 h before use). The solution was then diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 730 nm. The absorbance was measured at 30 min. Ascorbic acid was used as control. The ABTS radical-scavenging activity was calculated as = (Acontrol-Asample/Acontrol) × 100.

**Reducing power:** Reducing power was estimated by standard method given by Papuc et al. [41]. Briefly, samples (200 µL) were mixed with sodium phosphate buffer (pH 6.6), 1 mM $FeSO_4$, and 1% potassium ferricyanide and incubated for 20 min at 50°C after that trichloroacetic acid was added and the mixtures were centrifuged. Supernatant (2.5 mL) was mixed with an equal volume of water and 0.5 mL 0.1% $FeCl_3$. The absorbance was measured at 700 nm.

**Ferrous ion chelating assay:** For this, 1 mL of the sample (2-10 mg/mL) was mixed with 3.7 mL of ultrapure water, following which the mixture was reacted with ferrous chloride (2 mmol/L, 0.1 mL) and ferrozine (5 mmol/L, 0.2 mL) for 20 min. and the absorbance was read at 562 nm. EDTA was used as positive control. The chelating activity on the ferrous ion was calculated using the formula: chelating activity (%)=[($A_b$-$A_s$)/$A_b$] × 100, where $A_b$ is the absorbance of the blank and $A_s$ is the absorbance in the presence of the extract [42].

**Scavenging ability on superoxide anion radicals:** The scavenging activity of superoxide anion radicals was measured with standard method with minor modifications [43]. A tube containing polysaccharide sample (0-2.0 mg/mL, 1 mL) and Tris-HCl buffer (50.0 mM, pH 8.2, 3 mL) was incubated in a water bath at 25°C for 20 min and after this pyrogallic acid (5.0 mM, 0.4 mL) was added. HCl solution (8.0 M, 0.1 mL) was added to terminate the reaction after 4 min. The absorbance of the mixture was measured at 320 nm.

The scavenging ability of superoxide anion radicals was calculated using the following formula: scavenging ability (%)=(1–A sample/A control) × 100, where A control is the absorbance of control without the polysaccharide sample, and A sample is the absorbance in the presence of the polysaccharide sample.

**Ferric reducing antioxidant power (FRAP) assay:** The Ferric reducing antioxidant power was determined by standard method [44,45]. For this, FRAP reagent was prepared by mixing TPTZ (tripyridyltriazine) (2.5 mL, 10 mM in 40 mM HCl), 25 mL of 300 mM acetate buffer, and 2.5 mL of $FeCl_3.H_2O$ (20 mM). Freshly prepared FRAP reagent (1.8 mL) was taken in a test tube and incubated at 30°C in water bath for 10 minutes. Then, absorbance was taken at 0 min ($t_0$). Immediately, 100 µl of sample extract or standard and 100 µl of distilled water was added to the test tube, mixed and incubated at 30°C for 30 minutes. Then, the absorbance was taken at 593 nm ($t_{30}$). Ferrous sulphate was used as standard. The antioxidant potential of the sample extract was determined against a standard curve of ferrous sulphate and the FRAP value was expressed as µM $Fe^{2+}$ equivalents per gram of extract and calculated using the following equation:

FRAP value=Absorbance (sample+FRAP reagent) –Absorbance (FRAP reagent)

**Inhibition rate of peroxidation of polyunsaturated fatty acid from lipoprotein:** Inhibition rate of polyunsaturated fatty acid (PUFA) peroxidation was determined following a standard method described by Zhang et al. [46]. Briefly, yolk suspension was prepared with addition of fresh egg yolk to 0.1 M pH 7.4 phosphate buffers. Yolk suspension (0.2 mL) was mixed with 0.1 mL different concentration of polysaccharides (1-10 mg/mL). After that, 25 mM $FeCl_2$ (0.2 mL) and phosphate buffer (1.5 mL, 0.1 M, pH 7.4) were added to the mixture and incubated at 37°C for 15 min with continuous shaking. After this, TCA 20% (0.5 mL) was added to the mixture and centrifuged at 5,000 × g for 10 min. Supernatant (2 mL) was added with 1 mL 0.8% thiobarbituric acid, and the solution was incubated at 75°C for 10 min. The absorbance was determined at room temperature at 532 nm. The inhibition rate was calculated using the following formula: Inhibition rate %= [control-sample] / control.

## Experimental design

**One-factor-at-a-time:** In each experiment, one factor was varied, while all other factors were holding constant. Different carbon sources, nitrogen, mineral sources and different conditions were initially studied by single factor experiments in both of the species.

**Orthogonal matrix method:** To investigate the relationships between variables of medium components and optimize their concentrations for EPS and IPS production, the orthogonal matrix

experimental design $L_9$ ($3^4$) method was used.

## Chemicals

All the chemicals were purchased from Sigma Aldrich and all other unlabelled chemicals and reagents were of analytical grade.

## Results and Discussion

### Optimization of submerged culture conditions for polysaccharides production

To find the suitable medium capacities for maximum EPS and IPS production, *Isaria sinclairii* and *I. tenuipes* were grown in the media with different capacities. The maximum EPS (544.40 ± 1.76 mg/L) and IPS (340.12 ± 3.67 mg/L) production was observed in 150 mL of the medium, while least values of EPS (229.11 ± 1.40 mg/L) and IPS (202.10 ± 2.42 mg/L) production were observed in 50 mL of the medium. Similarly, submerged culture of *I. tenuipes* resulted maximum EPS (359.62 ± 1.81 mg/L) and IPS (164.20 ± 2.01 mg/L) production in the 150 mL medium and least values of EPS (220.11 ± 2.12 mg/L) and IPS (127.13 ± 1.49 mg/mL) were observed in 50 mL of the medium (Table 1). However, no significant difference ($p<0.05\%$) was observed in IPS production in the medium capacities from 150-200 mL in *I. tenuipes*. Variation in rotation speed in submerged cultures of *Isaria sinclairii* and *I. tenuipes* showed significant effect on EPS and IPS production. Submerged culture of *I. sinclairii* resulted maximum EPS (521.28 ± 1.18 mg/L) and IPS (321.16 ± 2.72 mg/L) production with rotation speed 150 rpm. Similar was observed in *I. tenuipes* with EPS (278.34 ± 1.10 mg/L) and IPS (174.21 ± 2.62) yield. Medium capacities showed significant effect on EPS and IPS production. Previous studies also revealed t he effect of medium capacities on EPS and IPS production and has been studied in other entomogenous fungi viz., *I. farinosa*, *Cordyceps ophioglossoides* and other ascomycetes [34,47].

Similar observations were made in *Cordyceps ophioglossoides*, in which maximum values for IPS production were obtained in the flasks containing 150 mL of medium and 150 rpm rotation speed [34]. Incubation time and pH range showed significant effect on EPS and IPS production in both *I. sinclairii* and *I. tenuipes* under submerged culture conditions. *I. sinclairii* culture incubated for 7 days showed high EPS

| Sources | *I. sinclairii* | | *I. tenuipes* | |
|---|---|---|---|---|
| **Medium capacity/ml** | **EPS** | **IPS** | **EPS** | **IPS** |
| 50 | 229.11 ± 1.40$^b$ | 202.10 ± 2.42$^b$ | 220.11 ± 2.12$^b$ | 127.13 ± 1.49$^a$ |
| 100 | 292.18 ± 3.30$^b$ | 244.14 ± 4.34$^b$ | 258.12 ± 3.47$^b$ | 158.13 ± 1.40$^a$ |
| 150 | 544.40 ± 1.76$^e$ | 340.12 ± 3.67$^c$ | 359.62 ± 1.81$^b$ | 164.20 ± 2.01$^a$ |
| 200 | 453.19 ± 2.10$^d$ | 254.18 ± 4.12$^b$ | 342.14 ± 345$^c$ | 161.62 ± 2.12$^a$ |
| 250 | 393.20 ± 1.40$^c$ | 243.22 ± 4.47$^b$ | 242.23 ± 2.49$^a$ | 149.13 ± 3.41$^a$ |
| **Rotation speed (rpm)** | | | | |
| 50 | 253.19 ± 2.15$^b$ | 203.11 ± 2.26$^b$ | 212.19 ± 2.32$^a$ | 112.10 ± 1.51$^a$ |
| 100 | 214.10 ± 4.74$^b$ | 214.13 ± 3.22$^b$ | 242.11 ± 2.13$^a$ | 144.26 ± 4.06$^a$ |
| 125 | 241.31 ± 3.26$^b$ | 241.13 ± 4.22$^b$ | 250.56 ± 2.21$^a$ | 159.20 ± 3.22$^a$ |
| 150 | 521.28 ± 1.18$^e$ | 321.16 ± 2.72$^c$ | 278.34 ± 1.10$^b$ | 174.21 ± 2.62$^b$ |
| 175 | 414.36 ± 3.19$^d$ | 309.33 ± 1.40$^c$ | 267.10 ± 3.12$^b$ | 166.12 ± 2.16$^b$ |
| 200 | 402.11 ± 4.14$^d$ | 285.21 ± 5.20$^b$ | 255.10 ± 3.15$^a$ | 152.13 ± 3.04$^a$ |
| **Incubation time/d** | | | | |
| 2 | 195.16 ± 2.17$^a$ | 90.00 ± 1.10 | 169.17 ± 2.19$^a$ | 63.11 ± 1.42$^a$ |
| 3 | 256.35 ± 3.19$^b$ | 163.12 ± 2.20$^a$ | 208.11 ± 3.11$^b$ | 108.21 ± 1.29$^a$ |
| 4 | 328.45 ± 2.23$^c$ | 182.12 ± 1.32$^a$ | 216.13 ± 4.15$^b$ | 119.10 ± 2.10$^a$ |
| 5 | 377.19 ± 1.11$^c$ | 187.19 ± 1.11$^a$ | 253.64 ± 3.17$^b$ | 127.42 ± 1.71$^a$ |
| 6 | 443.18 ± 3.14$^d$ | 194.04 ± 1.27$^a$ | 352.34 ± 2.20$^c$ | 166.15 ± 1.21$^a$ |
| 7 | 437.12 ± 2.91$^d$ | 167.42 ± 1.11$^a$ | 256.51 ± 3.50$^b$ | 154.12 ± 2.52$^a$ |
| 8 | 372.48 ± 3.71$^c$ | 142.25 ± 1.12$^a$ | 248.91 ± 5.15$^b$ | 147.10 ± 1.16$^a$ |
| 9 | 359.64 ± 4.30$^c$ | 140.14 ± 1.37$^a$ | 234.17 ± 2.16$^b$ | 131.20 ± 1.83$^a$ |
| 10 | 214.39 ± 1.92$^b$ | 104.32 ± 2.55$^a$ | 226.51 ± 2.15$^b$ | 120.12 ± 1.22$^a$ |
| **pH** | | | | |
| 3.0 | 261.10 ± 2.10$^c$ | 131.12 ± 1.17$^c$ | 205.15 ± 2.44$^a$ | 102.65 ± 1.19$^a$ |
| 4.0 | 298.14 ± 3.00$^c$ | 148.22 ± 1.20$^c$ | 244.22 ± 2.11$^a$ | 109.06 ± 1.71$^a$ |
| 5.0 | 365.35 ± 4.21$^c$ | 175.31 ± 2.25$^c$ | 264.34 ± 4.12$^a$ | 135.91 ± 1.94$^a$ |
| 6.0 | 385.48 ± 3.20$^c$ | 185.10 ± 1.27$^c$ | 242.54 ± 2.31$^b$ | 110.21 ± 1.52$^b$ |
| 7.0 | 343.61 ± 3.12$^c$ | 153.19 ± 2.32$^c$ | 212.21 ± 4.35$^a$ | 109.39 ± 1.62$^a$ |
| 8.0 | 262.37 ± 2.17$^b$ | 102.19 ± 1.72$^b$ | 182.14 ± 2.76$^a$ | 80.11 ± 2.85$^a$ |
| **Temperature** | | | | |
| 20 | 253.39 ± 2.28$^b$ | 153.30 ± 1.29$^a$ | 220.12 ± 2.10$^b$ | 125.11 ± 1.12$^a$ |
| 22 | 329.11 ± 2.18$^c$ | 215.16 ± 1.22$^b$ | 265.25 ± 2.33$^b$ | 137.10 ± 1.76$^a$ |
| 25 | 392.19 ± 2.20$^c$ | 222.11 ± 1.21$^b$ | 238.27 ± 2.10$^a$ | 120.41 ± 3.12$^a$ |
| 27 | 325.27 ± 3.20$^c$ | 215.18 ± 2.27$^b$ | 221.10 ± 4.40$^a$ | 119.19 ± 2.42$^a$ |
| 30 | 203.52 ± 3.12$^b$ | 237.13 ± 2.12$^b$ | 212.20 ± 2.80$^a$ | 116.21 ± 1.81$^a$ |
| 33 | 118.15 ± 3.10$^a$ | 108.16 ± 2.11$^a$ | 63.19 ± 1.32$^a$ | 028.19 ± 1.32$^a$ |
| Different letters represent the significant difference in each column and row ($p \leq 0.05$) | | | | |

**Table 1:** Effect of different factors on IPS production (mg/L) in submerged culture of *Isaria sinclairii* and *I. tenuipes*.

(437.12 ± 2.91 mg/L) and IPS (167.42 ± 1.11 mg/L) production. Under submerged conditions *I. sinclairii* resulted maximum EPS (385.48 ± 3.20 mg/L) and IPS (185.10 ± 1.27 mg/L) production was observed at pH 6.0 (Table 1). However, submerged culture of *I. tenuipes* showed maximum EPS (352.34 ± 2.20 mg/L) and IPS (166.15 ± 1.21 mg/L) production at incubation time of 6 days. Maximum EPS and IPS production was observed at pH 5.0 for this species (Table 1). Temperature 25°C and 22°C were observed ideal for EPS and IPS production in *I. sincalirii* and *I. tenuipes* respectively (Table 1). Present findings are in conformity with the results obtained in medicinal insect hosting fungi in which incubation period of 5-6 days and slightly acidic pH 5.0-6.0 promoted maximum IPS production [34,48]. The best temperature for polysaccharide production in *Cordyceps sinensis* as observed as 20°C and 25°C for *C. ophioglossoides* [49].

Eight different carbon sources were studied to find the suitable medium source for the production of EPS and IPS in *I. sinclairii* and *I. tenuipes*. Although, all the tested carbon sources yielded EPS and IPS in both of entomogenous species, but maximum EPS and IPS production took place in the medium supplemented with glucose as carbon source. The results are same as obtained for many entomogenous species of genus *Cordyceps* Fr. species, as glucose was found to be the most favourable carbon source for polysaccharide production [50,51]. To find the best nitrogen source, six different nitrogen sources were selected. Amongst them, peptone yielded maximum EPS (379.10 ± 2.35 mg/L) and IPS (244.18 ± 1.30 mg/L) in *I. sinclairii*. *I. tenuipes* yielded maximum EPS (274.15 ± 3.45 mg/L) and IPS (214.10 ± 1.41 mg/L) in the medium supplemented with yeast extract as nitrogen source. Previous studies have shown that supplementation of medium with nitrogen sources supported the production of polysaccharides under submerged culture conditions. Present results are in conformity with previous reports on *Cordyceps* and its anamorphic species [49]. Five different mineral sources were studied for EPS and IPS production. $NaH_2PO_4$ + $MgSO_4$ supported maximum EPS (374.86 ± 3.36 mg/L) and IPS production (279.80 ± 1.22 mg/L) in *I. sinclairii* whereas $NaH_2PO_4$ + $K_2HPO_4$ resulted maximum EPS (312.67 ± 1.21 mg/L) and IPS (256.40 ± 2.37 mg/L) in *I. tenuipes*. C/N ratio 10:1 promoted maximum EPS and IPS production in both species (Table 2). Similar results have been obtained for other entomogenous species like *Cordyceps ophiogllosoides*, as C/N ratio 10:1 provided maximum IPS (653.79 ± 5.24 mg/L) production [27]. Orthogonal experiments for the different factors on the yield of EPS and IPS showed a significant effect in both of the species. Results revealed the effect on EPS and IPS production in the order as: temperature>incubation time>pH>rotary speed>medium capacity (Tables 3 and 4).

Monosaccharide composition analysis of polysaccharides obtained from *I. sinclairii* and *I. tenuipes* showed glucose as chief component. However, xylose, rhamnose, mannose, galactose and fructose were also detected in small percentages (Table 5).

## Antioxidant activities of EPS and IPS

The DPPH scavenging activity of EPS and IPS extracted from the mycelium of *I. sinclairii* and *I. tenuipes* showed positive and direct

| Factors | | *I. sinclarii* | *I. tenuipes* | |
|---|---|---|---|---|
| | **EPS (mg/L)** | **IPS (mg/L)** | **EPS** | **IPS (mg/L)** |
| **Carbon sources** | | | | |
| Sucrose | 365.22 ± 1.14$^{c}$ | 234.23 ± 3.10$^{b}$ | 296.42 ± 1.48$^{b}$ | 246.42 ± 2.37$^{b}$ |
| Mannitol | 301.17 ± 3.15$^{c}$ | 221.16 ± 2.18$^{b}$ | 246.18 ± 5.17$^{b}$ | 188.42 ± 2.12$^{a}$ |
| Galactose | 287.10 ± 2.21$^{b}$ | 207.12 ± 3.29$^{b}$ | 213.10 ± 3.39$^{b}$ | 173.60 ± 1.34$^{a}$ |
| Starch | 211.20 ± 1.75$^{b}$ | 201.22 ± 2.57$^{b}$ | 235.32 ± 1.10$^{b}$ | 188.37 ± 2.11$^{a}$ |
| Glucose | 337.14 ± 1.37$^{c}$ | 267.19 ± 2.30$^{b}$ | 318.12 ± 2.32$^{b}$ | 208.15 ± 3.17$^{b}$ |
| Maltose | 242.11 ± 1.62$^{b}$ | 202.10 ± 3.74$^{b}$ | 157.52 ± 1.18$^{a}$ | 157.32 ± 2.19$^{a}$ |
| Fructose | 252.11 ± 2.19$^{b}$ | 212.11 ± 3.80$^{b}$ | 214.47 ± 3.33$^{a}$ | 119.34 ± 2.92$^{a}$ |
| Lactose | 172.46 ± 2.94$^{a}$ | 76.12 ± 3.17$^{a}$ | 174.10 ± 3.27$^{a}$ | 78.10 ± 3.27$^{a}$ |
| **Nitrogen source** | | | | |
| Yeast Extract | 322.89 ± 2.11$^{c}$ | 225.72 ± 1.71$^{b}$ | 274.15 ± 3.45$^{b}$ | 214.10 ± 1.41$^{b}$ |
| Peptone | 379.10 ± 2.35$^{c}$ | 244.18 ± 1.30$^{b}$ | 263.32 ± 2.63$^{b}$ | 201.19 ± 1.60$^{b}$ |
| $NaNO_2$ | 273.32 ± 2.76$^{b}$ | 204.90 ± 3.15$^{b}$ | 197.23 ± 2.43$^{b}$ | 149.11 ± 2.70$^{a}$ |
| $(NH4)_2SO_4$ | 224.33 ± 5.62$^{b}$ | 194.11 ± 1.17$^{a}$ | 194.52 ± 5.63$^{a}$ | 124.12 ± 1.60$^{a}$ |
| L – Arginine HCL | 209.10 ± 3.15$^{b}$ | 164.17 ± 2.42$^{a}$ | 187.24 ± 4.45$^{a}$ | 107.19 ± 2.46$^{a}$ |
| DL – Ascorbic Acid | 263.70 ± 2.16$^{b}$ | 203.11 ± 1.19$^{b}$ | 193.15 ± 2.26$^{a}$ | 103.12 ± 2.21$^{a}$ |
| **Mineral Sources** | | | | |
| $NaH_2PO_4$+$CaCl_2$ | 317.14 ± 6.11$^{c}$ | 212.19 ± 2.21$^{b}$ | 199.10 ± 6.17$^{a}$ | 169.61 ± 2.47$^{a}$ |
| $NaH_2PO_4$+$K_2HPO_4$ | 341.14 ± 2.45$^{c}$ | 211.25 ± 1.15$^{b}$ | 312.67 ± 1.21$^{c}$ | 256.40 ± 2.37$^{b}$ |
| $NaH_2PO_4$+$MgSO_4$ | 374.86 ± 3.36$^{c}$ | 279.80 ± 1.22$^{b}$ | 278.18 ± 3.63$^{b}$ | 248.22 ± 2.62$^{b}$ |
| $KH_2PO_4$+$CaCl_2$ | 334.89 ± 2.68$^{c}$ | 233.71 ± 2.22$^{b}$ | 245.37 ± 2.17$^{b}$ | 157.46 ± 1.46$^{a}$ |
| $KH_2PO_4$+$MgSO_4$ | 327.18 ± 3.17$^{c}$ | 217.11 ± 1.19$^{b}$ | 237.53 ± 4.23$^{b}$ | 137.30 ± 1.87$^{a}$ |
| **C/N ratio** | | | | |
| 40:1 | 117.16 ± 2.10$^{a}$ | 76.12 ± 1.11$^{a}$ | 82.29 ± 2.57$^{a}$ | 42.29 ± 3.12$^{a}$ |
| 30:1 | 294.19 ± 4.61$^{b}$ | 196.81 ± 4.61$^{a}$ | 142.20 ± 1.33$^{a}$ | 142.20 ± 3.10$^{a}$ |
| 20:1 | 313.11 ± 3.29$^{c}$ | 213.67 ± 2.21$^{b}$ | 153.72 ± 4.46$^{a}$ | 153.72 ± 1.23$^{a}$ |
| 10:1 | 388.19 ± 4.12$^{c}$ | 248.10 ± 4.12$^{b}$ | 281.64 ± 4.48$^{b}$ | 135.12 ± 2.13$^{a}$ |
| 5:1 | 334.84 ± 1.50$^{c}$ | 231.12 ± 2.28$^{b}$ | 219.15 ± 3.50$^{b}$ | 114.14 ± 1.34$^{a}$ |
| 1:1 | 293.72 ± 1.24$^{b}$ | 103.13 ± 1.14$^{a}$ | 192.29 ± 3.16$^{a}$ | 57.16 ± 1.35$^{a}$ |
| Different letters represent the significant difference in each column and row ($p \leq 0.05$) | | | | |

**Table 2:** Effect of different carbon, nitrogen, mineral sources and C/N ratio on EPS and IPS yield in submerged culture of *I. sinclairii* and *I. tenuipes*.

| Experimental group | Temperature (ºC) | pH | Rotary speed/r·min−1 | Culture time/d | EPS (mg/L) | IPS (mg/L) |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 312.13 ± 3.10 | 262.13 ± 2.62 |
| 2 | 1 | 2 | 2 | 2 | 370.21 ± 9.10 | 282.12 ± 11.15 |
| 3 | 1 | 3 | 3 | 3 | 380.20 ± 11.21 | 228.21 ± 12.16 |
| 4 | 2 | 1 | 2 | 3 | 578.23 ± 20.15 | 294.10 ± 24.19 |
| 5 | 2 | 2 | 3 | 1 | 562.60 ± 12.17 | 392.10 ± 19.23 |
| 6 | 2 | 3 | 1 | 2 | 642.65 ± 7.22 | 411.16 ± 10.52 |
| 7 | 3 | 1 | 3 | 2 | 242.12 ± 40.14 | 188.15 ± 12.31 |
| 8 | 3 | 2 | 1 | 3 | 322.10 ± 10.71 | 241.11 ± 12.70 |
| 9 | 3 | 3 | 2 | 1 | 220.10 ± 22.11 | 166.16 ± 12.10 |
| $K_1$ | 9.84 | 7.12 | 13.21 | 12.14 | | |
| $K_2$ | 19.12 | 12.14 | 13.42 | 14.21 | | |
| $K_3$ | 14.60 | 8.28 | 14.12 | 14.21 | | |
| R | 12.20 | 3.27 | 0.82 | 3.22 | | |
| $K_1$* | 440.60 | 325.46 | 444.11 | 318.42 | | |
| $K_2$* | 575.69 | 512.21 | 519.10 | 426.11 | | |
| $K_3$* | 298.13 | 332.20 | 358.20 | 425.17 | | |
| R* | 387.17 | 79.26 | 32.21 | 78.18 | | |

**Table 3:** Results obtained for orthogonal design *I. sinclarii.*

| Experimental group | Temperature (ºC) | pH | Rotary speed/r·min−1 | Culture time/d | EPS (mg/L) | IPS (mg/L) |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 221.12 ± 5.21 | 236.81 ± 2.62 |
| 2 | 1 | 2 | 2 | 2 | 322.11 ± 9.22 | 334.12 ± 11.12 |
| 3 | 1 | 3 | 3 | 3 | 311.12 ± 12.71 | 324.21 ± 11.14 |
| 4 | 2 | 1 | 2 | 3 | 398.10 ± 10.23 | 458.11 ± 32.14 |
| 5 | 2 | 2 | 3 | 1 | 391.61 ± 16.13 | 398.61 ± 12.11 |
| 6 | 2 | 3 | 1 | 2 | 442.61 ± 8.17 | 342.21 ± 4.15 |
| 7 | 3 | 1 | 3 | 2 | 178.10 ± 12.22 | 163.22 ± 12.13 |
| 8 | 3 | 2 | 1 | 3 | 262.12 ± 11.70 | 212.15 ± 8.15 |
| 9 | 3 | 3 | 2 | 1 | 176.25 ± 18.12 | 151.71 ± 11.23 |
| $K_1$ | 9.43 | 6.91 | 12.13 | 11.29 | | |
| $K_2$ | 12.16 | 10.15 | 10.13 | 13.52 | | |
| $K_3$ | 8.64 | 7.22 | 12.12 | 12.38 | | |
| R | 11.25 | 2.94 | 0.97 | 3.15 | | |
| $K^*_1$ | 325.31 | 310.18 | 314.12 | 322.41 | | |
| $K^*_2$ | 439.41 | 384.21 | 421.21 | 212.11 | | |
| $K^*_3$ | 242.13 | 241.20 | 311.13 | 362.11 | | |
| R* | 322.10 | 68.10 | 32.12 | 72.11 | | |
| $K_1$=Σ EPS at culture factor level 1/3., $K_1$*=Σ IPS yield at culture factor level 1/3 | | | | | | |

**Table 4:** Results obtained for orthogonal design *I. tenuipes.*

| Monosaccharides | *I. sinclairii* | *I. tenuipes* |
|---|---|---|
| Xylose | 15.17 ± 2.10 | 12.26 ± 5.19 |
| Glucose | 54.10 ± 6.13 | 44.67 ± 3.75 |
| Rhamnose | 32.11 ± 2.53 | 21.18 ± 4.57 |
| Mannose | 11.89 ± 3.75 | 25.80 ± 2.70 |
| Galactose | 0.10 ± 0.0 | 0.13 ± 0.01 |
| Fructose | 0.06 ± 0.0 | 0.01 ± 0.00 |
| Lactose | nd | nd |
| Maltose | nd | nd |
| nd=not detected | | |

**Table 5:** Monosaccharide composition of polysaccharides (%).

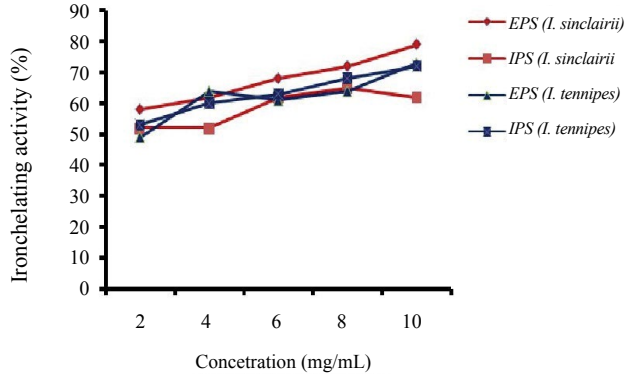


**Figure 2:** DPPH scavenging activity of EPS and IPS.

correlation with the concentration of the sample (Figure 2). The EPS showed higher DPPH scavenging activity than IPS. EPS and IPS extracted from both of species showed high DPPH scavenging activities. The results are also supported by high $EC_{50}$ values of both EPS and IPS (Table 6). DPPH radical scavenging activities of both these species were observed same as obtained for other medicinally important entomogenous species like *Cordyceps militaris* and *C. sinensis* [52,53]. The inhibition percentage of the ABTS radical by EPS and IPS of both

showed a direct positive relation with the concentration of the sample. The ABTS radical scavenging activity of IPS was found to be higher as compared to EPS in both of species (Figure 3). At a concentration of 10.0 mg/mL, the percentage inhibition of EPS and IPS was found to be maximum and showing their ability to quench the free radicals in the system. The results indicated that the EPS and IPS of both entomogenous species possessed significant scavenging power for the ABTS radicals. However EPS showed higher ABTS radical scavenging activity than IPS (Figure 3).

The results obtained for reducing power abilities of EPS and IPS in submerged culture showed that both types of polysaccharides possess significant reducing capacities. The reducing powers of EPS and IPS increased as the sample concentration increased (Figure 4). The reducing power of EPS was found to be higher than reducing power of IPS in both of the species. However at highest concentration 10 mg/mL, the reducing power of EPSs of both species was found to be same (1.11 ± 0.01 mg/mL). Reducing power of EPSs was found to be higher as compared to IPSs in both of the species (Figure 4). The results are further supported by $EC_{50}$ values. Present results showed that EPS and IPS of both of these species contained reductones which react with certain precursors of peroxides to prevent peroxide formation. The iron chelating ability of the EPS and IPS was found to be related with the concentration of sample. At initial concentrations 2 mg/mL, there was not much difference observed between the iron chelating ability of both EPS and IPS (49-54%). However, at higher sample concentrations EPS showed higher iron chelating ability than IPS in both of the species (Figure 5). EPSs showed maximum iron chelating activity in both of the species at concentration 10 mg/mL. The results further supported by $EC_{50}$ values (Table 6). EPS and IPS of both of the species showed high scavenging ability on superoxide anion radicals. At high concentration the scavenging ability was found to be maximum (Figure 6). *I. sinclairii* showed higher values for scavenging radicals than *I. tenuipes* however at concentration 8 mg/mL, EPSs of both species possessed same activity (Figure 6). EPS and IPS of both species showed high FRAP activity. The results are supported by $EC_{50}$ values (Table 6). Experiments were performed to study the inhibition rate of polyunsaturated fatty acids. Present investigations showed that EPS as well as IPS of both species showed high inhibition rate of polyunsaturated fatty acids. At the highest polysaccharide concentration the inhibition rate was found to be high (Figure 7).

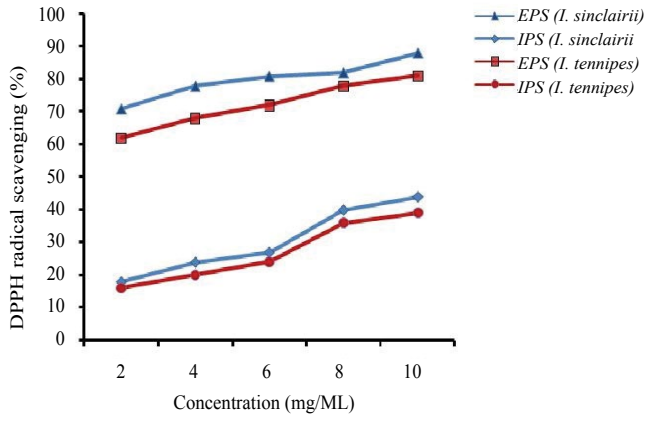


**Figure 3:** ABTS radical scavenging activity of EPS and IPS.

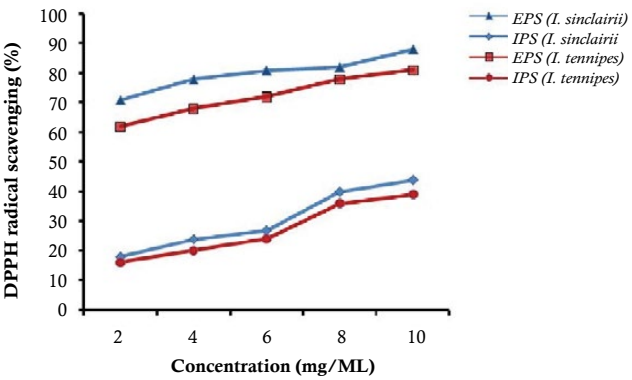


**Figure 4:** Reducing power of EPS and IPS.

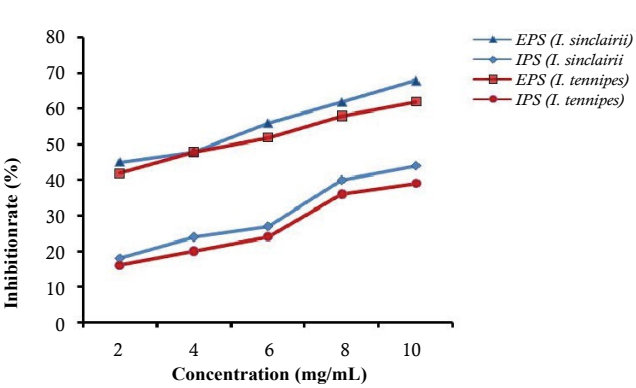


**Figure 5:** Iron chelating activity of EPS and IPS.

## Conclusion

Submerged culture of *I. sinclairii* and *I. tenuipes* required several factors for the production of EPS and IPS. Factors such as effect of temperature, rotation speed, pH, incubation time, carbon, nitrogen,

| $EC_{50}$ | *I. sinclairii* (mg/mL) | | *I. tenuipes* (mg/mL) | |
|---|---|---|---|---|
| | EPS | IPS | EPS | IPS |
| DPPH radical scavenging activity | 7.52 ± 0.10 | 3.17 ± 0.20 | 6.12 ± 0.10 | 2.71 ± 0.14 |
| ABTS radical scavenging activity | 6.29 ±0.13 | 4.25 ± 0.19 | 5.43 ±0.13 | 3.11 ± 0.19 |
| Reducing power | 7.17 ± 0.22 | 3.11 ± 0.17 | 6.36 ± 0.22 | 2.43 ± 0.27 |
| Iron chelating activity | 2.04 ± 0.31 | 1.24±0.16 | 1.84 ± 0.31 | 0.85 ± 0.31 |
| FRAP | 1.76 ± 0.05 | 1.51 ± 0.04 | 1.51 ± 0.04 | 1.51 ± 0.04 |
| Scavenging activity superoxide ion | 2.14 ± 0.20 | 1.74 ± 0.10 | 1.88 ± 0.30 | 0.75 ± 0.11 |

**Table 6:** $EC_{50}$ value of EPS and IPS.

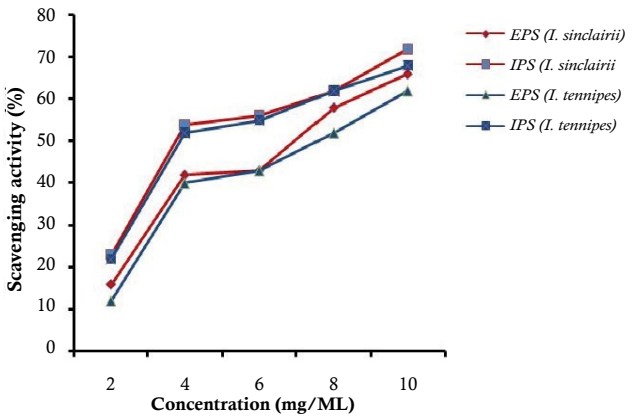


**Figure 6:** Scavenging ability on superoxide anion radicals.

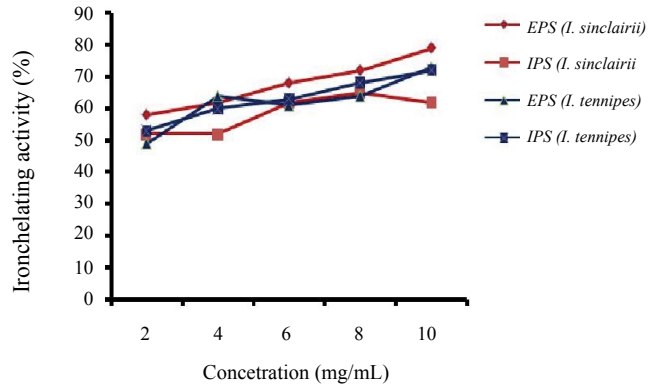


**Figure 7:** Inhibition rate of peroxidation of polyunsaturated fatty acid from lipoprotein.

mineral sources, and carbon to nitrogen ratio showed significant effects on the production of EPS and IPS. Composition analysis of polysaccharides obtained from *I. sinclairii* and *I. tenuipes* showed glucose as chief component. As observed in the present studies EPS and IPS of both species exhibited significant DPPH radical scavenging activities, ABTS radical scavenging activities, reducing power, Iron chelating activities, scavenging activities of superoxide anion radicals, ferric reducing antioxidant power, as well as inhibition rate of peroxidation of polyunsaturated fatty acid from lipoprotein. Due to high antioxidant potential of EPS and IPS, present studies will be helpful for its large scale industrial fermentations and commercial use like other useful ascomycetes species namely *Cordyecps sinensis, C. militaris* and *C. ophioglossoides*.

## Acknowledgement

Author wish to thank Science and Engineering Board, Department of Science and Technology, New Delhi for research grant under Young Scientist Scheme (SB/FT/LS-04/2013) to carry out present studies.

## References

1. Humber RA, Hansen KS, Wheeler MM (2011) Isaria plus Paecilomyces, Purpureocillium, and Evlachovaea, ARSEF Catalog.
2. Kirk PM, Cannon P, David JC, Stalpers JA (2008) Ainsworth and Bisby dictionary of the fungi. (10th ed) UK: CAB International, Wallingford, Oxon.
3. Kobayasi Y (1982) Keys to the taxa of the genera Cordyceps and Torrubiella. Trans Mycol Soc Japan 23: 329-364.
4. Chen YJ, Shiao MS, Lee SS, Wang SY (1997) Effect of Cordyceps sinensis on the proliferation and differentiation of human leukemic U937 cells. Life Sci 60: 2349-2359.
5. Kiho T, Yamane A, Hui J, Usui S, Ukai S (1996) Polysaccharides in fungi, XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of *Cordyceps sinensis* and its effect on glucose metabolism in mouse liver. Biol Pharm Bull 19: 294-296.
6. Ji YB (1999) Pharmacological actions and applications of anitcancer traditional chinese medicines Cordycepssinensis (Berk) Sacc. Heilongjiang Ke-Xue-Ji-Shu Chu-Ban-She, Haerbin, China (in Chinese) 494-501.
7. Ji DB, Ye J, Li CL, Wang YH, Zhao J, et al. (2009) Anti-aging effect of *Cordyceps sinensis* extract. Phytother Res 23: 116-122.
8. Zhao CS, Yin WT, Wang JY, Zhang Y, Yu H, et al. (2002) CordyMax Cs-4 improves glucose metabolism and increases insulin sensitivity in normal rats. J Altern Complement Med 8: 309-314.
9. Mizuno T (1999) Medicinal effects and utilization of Cordyceps (Fr.) Link (Ascomycetes) and Isaria Fr. (Mitosporic fungi) Chinese caterpillar fungi Tochukaso. Int J Med Mushrooms 1: 251-262.
10. Kim SW, Hwang HJ, Xu CP, Na YS, Song SK, et al. (2002) Influence of nutritional conditions on the mycelial growth and exopolysaccharide production in *Paecilomyces sinclairii.* Lett Appl Microbiol 34: 389-393.
11. Hsu CH, Sun HL, Sheu JN, Ku MS, Hu CM, et al. (2008) Effects of the immunomodulatory agent *Cordyceps militaris* on airway inflammation in a mouse asthma model. Pediatr Neonatol 49: 171-178.
12. Zhu SJ, Pan J, Zhao B, Liang J, Ze-Yu W, et al. (2013) Comparisons on enhancing the immunity of fresh and dry *Cordyceps militaris in vivo* and *in vitro*. J Ethnopharmacol 149: 713-719.
13. Lin XX, Xie QM, Shen WH, Chen Y (2001) Effects of fermented Cordyceps powder on pulmonary function in sensitized guinea pigs and airway inflammation in sensitized rats. Zhongguo Zhong Yao Za Zhi 26: 622-625.
14. Yu RM, Yin Y, Yang W, Ma WL, Yang L, et al. (2009) Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured *Cordyceps militaris*. Carbohydr Polym 75: 166-170.
15. Liu JL, Fei Y (2008) Enhancement of *Cordyceps taii* polysaccharide and *Cordyceps pruinosa* polysaccharide on cellular immune function *in vitro*. J Immunol 17: 189-191.
16. Li FH, Liu P, Xiong WG, Xu GF (2006) Effects of corydyceps polysaccharide on liver fibrosis induced by DMN in rats. Zhongguo Zhong Yao Za Zhi 31: 1968-1971.
17. Chen J, Zhang W, Lu T, Li J, Zheng Y, Kong L (2006) Morphological and genetic characterization of a cultivated *Cordyceps sinensis* fungus and its polysaccharide component possessing antioxidant property in H22 tumour-bearing mice. Life Sci 78: 2742-2748.
18. Kim HO, Yun JW (2005) A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures. J Appl Microbiol 99: 728-738.
19. Yang J, Zhang W, Shi P, Chen J, Han X, et al. (2005) Effects of exopolysaccharide fraction (EPSF) from a cultivated *Cordyceps sinensis* fungus on c-Myc, c-Fos, and VEGF expression in B16 melanoma-bearing mice. Pathol Res Pract 201: 745-750.
20. Koh JH, Kim JM, Chang UJ, Suh HJ (2003) Hypocholesterolemic effect of hot-water extract from mycelia of *Cordyceps sinensis*. Biol Pharm Bull 26: 84-87.
21. Yu R, Song L, Zhao Y, Bin W, Wang L, et al. (2004) Isolation and biological properties of polysaccharide CPS-1 from cultured *Cordyceps militaris*. Fitoterapia 75: 465-472.
22. Hsu TH, Shiao LH, Hsieh C, Chang DM (2002) A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom Dong Chong Xia Cao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. Food Chem 78: 463-469.
23. Li SP, Zhao KJ, Ji ZN, Song ZH, Dong TTX, et al. (2003) A polysaccharide isolated from *Cordyceps sinensis*, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. Life Sci 73: 2503-2513.
24. Sharma SK, Gautam N, Atri NS (2015) Evaluation of mycelial nutrients,

bioactive compounds, and antioxidants of five Himalayan entomopathogenic ascomyceteous fungi from India. Int J Med Mushrooms 7: 661-669.

25. Meng F, Liu X, Jia L, Song Z, Deng P, et al. (2010) Optimization for the production of exopolysaccharides from *Morchella esculenta* SO-$O_2$ in submerged culture and its antioxidant activities *in vitro*. Carbohydr Polym 79: 700-704.

26. Huang BM, Ju SY, Wu CS, Chuang WJ, Sheu CC, et al. (2001) *Cordyceps sinensis* and its fractions stimulate MA-10 mouse Leydig tumor cell steroidogenesis. J Androl 22: 831-837.

27. Vinhal Costa Orsine J, Carvalho Garbi Novaes MR, Ramírez Asquieri E (2012) Nutritional value of *Agaricus sylvaticus*: mushroom grown in Brazil. Nutr Hosp 27: 449-455.

28. Lin XX, Xie QM, Shen WH, Chen Y (2001) Effects of fermented Cordyceps powder on pulmonary function in sensitized guinea pigs and airway inflammation in sensitized rats. Zhongguo Zhong Yao Za Zhi 26: 622-625.

29. Yu RM, Yin Y, Yang W, Ma WL, Yang L, et al. (2009) Structural elucidation and biological activity of a novel polysaccharide by alkaline extraction from cultured *Cordyceps militaris*. Carbohydr Polym 75: 166-170.

30. Hai Bang T, Suhara H, Doi K, Ishikawa H, Fukami K, et al. (2014) Wild Mushrooms in Nepal: Some Potential Candidates as Antioxidant and ACE-Inhibition Sources. Evid Based Complement Alternat Med 2014: 195305.

31. Abdullah N, Ismail SM, Aminudin N, Shuib AS, Lau BF (2012) Evaluation of Selected Culinary-Medicinal Mushrooms for Antioxidant and ACE Inhibitory Activities. Evid Based Complement Alternat Med 2012: 464238.

32. Huang HC, Liu YC (2008) Enhancement of polysaccharide production by optimization of culture conditions in shake flask submerged cultivation of *Grifola umbellate*. J Chin Inst Chem Eng 39: 307-311.

33. Pokhrel CP, Ohga S (2007) Submerged culture conditions for mycelial yield and polysaccharides production by *Lyophyllum decastes*. Food Chem 105: 641-646.

34. Qinqin XU, Zhenhua LIU, Yisheng SUN, Zhongjie DING., Longxian LU, et al. (2012) Optimization for Production of Intracellular Polysaccharide from *Cordyceps ophioglossoides* L2 in Submerged Culture and its Antioxidant Activities in vitro. Biotechnology and Bioengineering 20: 294-301.

35. Papagianni M (2004) Fungal morphology and metabolite production in submerged mycelial processes. Biotechnol Adv 22: 189-259.

36. Fang QH, Zhong JJ (2002) Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites-anoderic acid and polysaccharide. Biochem Eng J 10: 61-65.

37. Sharma SK, Gautam N, Atri NS (2015) Optimization, Composition, and Antioxidant Activities of Exo- and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu and Mont. Evid Based Complement Alternat Med 2015: 462864.

38. Atri N, Sharma SK, Joshi R, Gulati A, Gulati A (2013) Nutritional and neutraceutical composition of five wild culinary-medicinal species of genus Pleurotus (higher Basidiomycetes) from northwest India. Int J Med Mushrooms 15: 49-56.

39. Vamanu E (2012) Biological activities of the polysaccharides produced in submerged culture of two edible *Pleurotus ostreatus* mushrooms. J Biomed Biotechnol 2012: 565974.

40. Li PL, Huo W, Su W, Lu R, Deng C, et al. (2011) Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. J Serb Chem Soc 76: 709-717

41. Papuc C, Crivineanu M, Goran G, Nicorescu V, Durdun N (2010) Free radicals scavenging and antioxidant activity of European mistletoe (*Viscum album*) and European birthwort (*Aristolochia clematitis*). Revista de Chimie 61: 619-622.

42. Oyetayo VO, Dong CH, Yao YJ (2009) Antioxidant and antimicrobial properties of aqueous extract from *Dictyophora indusiata*. T O Myc J 3: 20-26.

43. Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47: 469-474.

44. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239: 70-76.

45. Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem 53: 1841-1856.

46. Zhang EX, Yu LJ, Zhou YL, Xiao X (1996) Studies on the Peroxidation of Polyunsaturated Fatty Acid from Lipoprotein Induced by Iron and The evaluation of The Anti-oxidative Activity of Some Natural Products. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai) 28: 218-222.

47. Jiang YH, Jiang XL, Wang P, Mou HJ, Hu XK, et al. (2008) The antitumor and antioxidative activities of polysaccharides isolated from *Isaria farinosa* B05. Microbiol Res 163: 424-430.

48. Hsieh C, Tsai MJ, Hsu TH, Chang DM, Lo CT (2005) Medium optimization for polysaccharide production of *Cordyceps sinensis.* Appl Biochem Biotechnol 120: 145-157.

49. Dong CH, Yao YJ (2005) Nutritional requirements of mycelial growth of *Cordyceps sinensis* in submerged culture. J Appl Microbiol 99: 483-492.

50. Kim SY, Park SK, Park HK, Kim SW (1994) Compositional sugar analysis of antitumor polysaccharides by high performance liquid chromatography and gas chromatography. Arch Pharm Res 17: 337-342.

51. Yu KW, Suh HJ, Bae SH, Lee CS, Kim SH, et al. (2001) Chemical properties and physiological activities of stromata of *Cordyceps militaris*. J Microbiol Biotechnol 11: 266-274.

52. Gu YX, Song YW, Fan LQ, Yuan QS (2007) Antioxidant activity of natural and cultured *Cordyceps* sp. Zhongguo Zhong Yao Za Zhi 32: 1028-1031.

53. Yuan X, Sun H, Liu Y, Shiroshita T, Kawano S, et al. (2014) Anti-cancer activity comparisons of aqueous extracts from I*nonotus obliquus, Cordyceps militaris* and *Uncaria tomentosa* in *vitro* and *in vivo*. J Pharmacogn Phytochem 2: 19-25.