Antioxidant activity and cytotoxicity of exopolysaccharide from mushroom *Hericium coralloides* in submerged fermentation

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Abstract
Mushrooms of the genus *Hericium* spp. represent a series of delicious edible mushrooms with medicinal value. Here, for the first time, the species native to Iran, the mushroom *Hericium coralloides*, was collected in Mazandaran province, identified, and registered with the NCBI under accession number MW136052. The production of exopolysaccharides (EPS) in submerged culture was optimized using the response surface method. Among the physicochemical and culture medium conditions tested, rotation speed and concentration of maltose and peptone of soybean significantly affected the production of EPS. The proposed model predicts maximum EPS production (0.13 g/L) at 50 g/L maltose, 3 g/L soy peptone, and 1 g/L yeast extract, pH = 6.5, 200 rpm, inoculum at 5% v/v, and 22 °C. The molecular weight of the EPS chains was 413 and 1578 Da. EPS has antioxidant action (EC50 = 6.59 mg/mL) and cytotoxic activity against cancer cells. The viability of AGS and MKN-45 cancer cell lines declined to 20 and 30% after 48 h of the EPS treatment. *H. coralloides* EPS could be considered a natural dietary anti-cancer supplement. Further studies are necessary to understand the mechanism of the *H. coralloides* EPS activity on the cell cycle of cancer cells and to prove its action in vivo.

Keywords *Hericium coralloides* · Antioxidant · Anticancer · Optimization · Submerged culture · Exopolysaccharide · MW136052

1 Introduction

In recent decades, fungi have attracted the attention of researchers due to their various nutritional and medicinal activities [1, 2]. Currently, 110,000 species of fungi have been described. Studies show that only 10% of the world’s fungal biodiversity is known [3, 4]. Fungi contain a wide range of powerful agents with medicinal properties. From ancient times, the fungi, especially mushrooms, have been considered a delicious food worldwide due to their unique taste [5, 6]. Also, they are prebiotic and about 50% of edible mushrooms were considered functional food [7, 8]. Recent studies have shown that the active compounds of fungi can be used as dietary supplements. These compounds can be divided into high molecular weight polymers, including polysaccharides, proteoglycans, and glycoproteins, etc., and secondary metabolites (acids, terpenoids, phenolics, alkaloids, lactones, sterols, nucleotide analogs, and vitamins). Mushrooms belonging to the Basidiomycota division are a rich source of bioactive compounds that can be used as dietary supplements [9–12]. Edible fungi, especially basidiomycetes, have functional properties that include immunomodulatory and anti-cancer activity, which have been explored and reported in recent reports [13]. Anti-COVID and gene expression regulatory properties have been reported, and show that polysaccharides satisfactorily modulate the immune system, helping to preserve cellular integrity and homeostasis [14, 15].
One of the most widely used fungi in traditional East Asian medicine is the medicinal fungus *Hericium*. The genus *Hericium* taxonomically belongs to the division Basidiomycota, class Agaricomycetes, order Russulales, and family Hericiaceae. To date, 53 species of this fungus are known worldwide [16, 17]. The fungus is more common in Japan, North America, and Eurasia. The most famous species of this fungus is *Hericium erinaceus* (lion’s mane). This fungus has long been used to treat cancer and strengthen the immune system [18, 19]. Recent studies, however, show that the fungus induces the expression of nerve growth factors, improves the function of the nervous system and the brain, and can play a role in preventing and improving diseases such as Parkinson’s and Alzheimer’s [20, 21]. The effectiveness of metabolites of this fungus in the treatment of depression has also been studied clinically [22]. One of the lesser known *Hericium* species is *Hericium coralloides*, which is a rare species in European and Asian countries [23, 24]. In recent years, the antioxidant activity of this fungus [25] and its growth in co-cultivation with *Fomes fomentarius* and *Schizophyllum commune* have been studied [26]. According to our research, detailed information on the optimal growth conditions for polysaccharide production of this fungus is not yet available.

Studies show that the physicochemical conditions of the culture medium can have a significant effect on the growth and production of fungal metabolites. Gerbec et al. (2015) compared the growth and production of *H. erinaceus* metabolites in a culture media, containing different concentrations of the peptone casein and NaCl. The highest growth rate was detected in a medium containing 0.56% w/v NaCl and 3.4% w/v casein peptone [27]. Malinowska et al. (2009) used the central composite rotatable design to optimize the production of biomass and polysaccharide of *H. erinaceus*. Various factors, including sources of carbon and nitrogen, vitamins, minerals, and initial pH, were examined. Under optimal culture conditions, the maximum biomass production was 14.44 g/L, 1.85 times higher than in the initial medium [28]. Huang et al. (2007) studied nutritional needs for biomass and EPS production by *H. erinaceus* CZ-2. The maximum biomass yield and EPS was 16.07 g/L and 1.314 g/L, respectively [29].

Following the work of our research group in optimizing the production of metabolites of medicinal fungi and investigating their biological activity, three main goals are pursued in this research: (1) identification of *H. coralloides* collected from the forests of Mazandaran, Iran; (2) determining the optimal culture media and conditions for producing of EPS of this fungus; and (3) investigating the antioxidant and anti-cancer properties of *H. coralloides* EPS.

## 2 Material and methods

### 2.1 Materials

2.2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Germany). PCR mixtures were purchased from Cinnagen (Iran). The cell culture media and MTT kit was purchased from Bio-Idea (Iran) and the rest of the chemicals were purchased from Merck (Germany).

### 2.2 Sampling, identification, and culture of *H. coralloides*

Healthy fruiting bodies of the *H. coralloides* were harvested in the forest of Mazandaran Province (Iran) using a random sampling method based on the morphological characteristics.

The collected samples were identified microscopically after staining with lactophenol cotton blue and using molecular methods. The selected sample was cultured on potato dextrose agar (PDA) in a petri dish. After 4 days of cultivation (Binder, USA) at 25 °C, 1 × 1 cm² of cultivated fungus was transferred to potato dextrose broth (PDB) medium, and incubated further at 25 °C at 150 rpm. After 5 days, mycelial mass was used for molecular analysis.

### 2.3 DNA amplification and sequence analysis

The mycelial mass of mushrooms was pulverized with liquid nitrogen. Then 600 μL of CTAB buffer was added to the mycelium and incubated at 65 °C. After 45 min, 600 μL of the chloroform–isoamyl alcohol mixture was added at a ratio of 1:24 and centrifuged at 10,000 g for 10 min. DNA-containing supernatant was combined with isopropanol (− 20 °C) and kept at −20 °C for 30 min. The DNA precipitate was separated by centrifugation (15 min at 10,000 g), washed with 70% ethanol, lyophilized, and stored at −20 °C.

Amplification of rDNA fragments was performed using a mixture of 10×PCR buffer (50 mM), MgCl₂ (10 mM), dNTP, primers, and Taq DNA polymerase. ITS4 (5′TCC TCGCTTATTGTAGTGC-3′) and ITS5 (5′GGAAGTAAA AGTCGTAACAAGG-3′) primers were used to identify ITS5 gene. The obtained PCR product was sent to Takapouzist company (Iran) for sequencing, and the result of sequences was examined in the GenBank (http://www.ncbi.nlm.nih.gov). The obtained sequences were reviewed with Bio Edit software (v7.1.9) and compared with database entries using the blast search program.

For phylogenetic analysis, the ITS5 gene sequence of MW136052.1 with other isolates was registered in the gene bank by the maximum likelihood method. The numbers
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recorded at the branches indicate the percentage of cluster confirmation with 1000 replications (bootstrap). Molecular Evolutionary Genetics Analysis (MEGA6) software also used for analyses of DNA sequences.

### 2.4 H. coralloides EPS extraction and characterization

After 7 days of fungal growth in liquid medium, biomass was separated and the supernatant was mixed with ethanol (96%) in a ratio of 1:4 v/v and stored at 4 °C. After 24 h, centrifugation at 11,000 g for 20 min was performed to separate the EPS. The EPS was then washed with ethanol (75%) and centrifuged at 11,000 g for 20 min again. In the last stage, the EPS was lyophilized and stored at −20 °C.

The EPS molecular weight was determined by Shimadzu LC-20A gel permeation chromatography (GPC) using Ultrahydrogel 250 column (7.8×300 mm). Briefly, 20–50 µl of 2 mg/ml EPS dissolved in distilled water was injected. Elution was carried out with 0.1 M sodium nitrate at a flow rate of 1 ml/min.

Dried polysaccharide was used for structural analysis. The Fourier-transform infrared spectroscopy (FTIR) spectra of EPS was used to investigate the functional groups and structure in a 400–4000 cm⁻¹ spectral range (Thermo, AVATAR, US).

X-ray diffraction patterns (XRD) was performed to determine the crystallinity structure of EPS (Philips PW1730, Netherlands). In the normal XRD test, the scanning angle is from 5 to 130°.

### 2.5 Optimization of parameters affecting the H. coralloides EPS production

#### 2.5.1 Physicochemical condition

Four physicochemical factors most affecting fungal growth and EPS production were selected: pH, temperature, stirring rate, and inoculation rate (Table 1). The variables were examined at three levels using Design-Expert v11 software and the D-optimal response surface method. For the test, 150 mL PDB medium was prepared in a 250-mL Erlenmeyer flask, inoculated with 7-day-old H. coralloides culture, and incubated on a shaker for 7 days according to experiment conditions followed by EPS extraction [29, 30].

#### 2.5.2 Culture medium components

Three culture medium parameters affecting EPS yield were checked so far: maltose, yeast extract, and soy peptone (Table 2). The best culture composition for the H. coralloides EPS production was studied by the response surface method (RSM) using central composite design (CCD). Since no specific composition was observed for the production of H. coralloides EPS, the culture medium was selected and optimized according to the initial experiments and previous studies [27–29]. All test steps were performed as above.

### 2.6 Biological assessment

#### 2.6.1 Antioxidant assay

The antioxidant potential of EPS was tested with DPPH radical scavenging assay as described previously. One milliliter of 0.2 mM DPPH methanol solution was mixed with 4 mL of EPS solution at different concentrations or with butylated hydroxytoluene (BHT) as a positive control at a 2 mg/mL concentration. The samples were then gently shaken and incubated in the dark for 30 min followed by measurement of absorbance at 517 nm with a microplate reader (Carry 100 Bio, Varian, Australia).

#### 2.6.2 MTT assay

EPS cytotoxicity was tested using MTT assay on MKN-45 (human gastric adenocarcinoma) and AGS (human Caucasian gastric adenocarcinoma) cancer cell lines (Iran Biological Resource Center, Tehran, Iran). Cell lines were maintained in RPMI 1640 medium, containing 2 mM l-glutamine and 10% FBS in a cell incubator at 37 °C and 5% CO₂. A total of 10,000 cells were seeded in each well of 96-wells plate for 24 h. EPS solution was added to each well in a certain concentration (25 to 1000 µg/mL) and after 24 h, the MTT test was performed and calculated according to the manufacturer’s protocol.

### 2.7 Statistical analysis

Data are presented as mean ± SD of at least three independent experiments. Sample size (n) indicates the number of replications. Statistical analysis was performed with the Design-Expert v11 software package (Stat-Ease, USA) and Microsoft Excel 2016, version 16.0. The significant differences between the samples were determined using a one-way ANOVA. p values below 0.05 (p < 0.05) were considered to be statistically significant.

### 3 Results

#### 3.1 H. coralloides identification

Morphological observation showed this fungus fruiting body was 5–60 cm with interconnected branches distributed on the coralloid branches' surface (Fig. 1a). The spore size was 3.5×3.4 μm and almost round and white. Microscopic
images showed that the entire surface of the fungus was covered with a layer containing basidia mixed with cystidia (Fig. 1b, c). This fungus was identified as *H. coralloides* with 90% similarity to *H. coralloides* MT044405.1 and was registered in the NCBI database under the access number MW136052 (Fig. 1d).

### 3.2 Optimization of physicochemical condition affecting the *H. coralloides* EPS production

The results of optimizing the physicochemical conditions of the culture medium to produce the EPS are shown in the Table 1. $R^2$ in this experiment was 90% (adjusted $R^2 = 80$%), which indicates a good correlation coefficient and shows the agreement of experimental data and model. Among studied physicochemical variables, the rotation speed had significant effects ($p < 0.05$) on the production of *H. coralloides* EPS (Table S1).

Based on the calculated value of regression coefficients, the following equation is proposed.

$$\text{EPS} = 0.0289 + 0.0083A - 0.0078B + 0.0168C - 0.0015D$$  \hfill (1)

In this equation, $A$ is the pH, $B$ is the temperature, $C$ is the rotation, and $D$ is the inoculum.

Figure 2 shows the effect of physicochemical variables in their different combinations on EPS production. With the rotation speed of 150 rpm and the inoculation rate of 10% v/v, the optimal points for the EPS production were at pH = 6.5 and 22 °C (Fig. 2a). By increase to 200 rpm rotation speed (Fig. 2a), EPS production reached 0.06 g/L compared to 0.04 g/L on the previous graph. Finally, the effect of the inoculation rate on EPS production was investigated (Fig. 2c). It was observed that the inoculation rate had little effect on EPS production, and its optimum amount was 5% v/v. Thus, the optimum culture condition for the production of *H. coralloides* EPS was at pH = 6.5, 200 rpm, 5% v/v inoculation, and 22 °C, and EPS production reached 0.0633 g/L.

### 3.3 Optimization of culture medium components affecting the *H. coralloides* EPS production

According to our preliminary experiments and other studies, the culture medium based on maltose was selected as the best source of carbon, yeast extract as a nutritional source, and soy peptone as a source of nitrogen. According to the data observed in this study, all cultures were performed at pH = 6.5, 22 °C, rotation speed 200 rpm, and 5% v/v inoculation of 7-day seed culture.

Table 2 shows the impact of the experimental conditions on EPS yield. The $R^2$ value was 87% (adjusted $R^2 = 81$%), indicating a good agreement between the experimental data and the regression model. According to Table S2, maltose (A), and soy peptone (C), as well as AC combination had significant effects on EPS production. The EPS production equation is as follows:

$$\text{EPS} = 0.0644 + 0.0236A + 0.0002B + 0.0207C - 0.0105 AB + 0.0165 AC - 0.0003BC$$  \hfill (2)

In this equation, $A$ is the maltose, $B$ is the yeast extract, and $C$ is the soy peptone.

![Fig. 1 Characteristics of *H. coralloides* isolated from Mazandaran forests. Fruiting body of *H. coralloides* (a), optical microscope image of fungal hyphae with lactophenol cotton blue staining (b), and image of basidia and glostidia of this fungus (c). Phylogeny of *H. coralloides*, reconstructed from the ITS dataset (d)](image-url)
The following 3D contour shows (Fig. 2d) that by decreasing the yeast extract concentration and increasing the maltose concentration, given the median limit for soy peptone, EPS production increased (0.09 g/L). By increasing soy peptone concentration to 3 g/L, the production of EPS reached 0.12 g/L (Fig. 2e). In our experimental conditions, the optimum culture media contains 50 g/L maltose, 3 g/L soy peptone, and 1 g/L yeast extract. EPS production in this medium reached 0.13 g/L.

### Table 1  EPS yield in response to different physicochemical factors

| Run | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Response EPS (g/L) |
|-----|----------|----------|----------|----------|-------------------|
| 1   | 5.5      | 28       | 100      | 10       | 0.010             |
| 2   | 5.5      | 22       | 150      | 15       | 0.028             |
| 3   | 4.5      | 28       | 200      | 15       | 0.024             |
| 4   | 6.5      | 28       | 150      | 5        | 0.024             |
| 5   | 6.5      | 25       | 100      | 15       | 0.020             |
| 6   | 5.5      | 25       | 200      | 5        | 0.048             |
| 7   | 6.5      | 22       | 200      | 10       | 0.080             |
| 8   | 4.5      | 22       | 100      | 5        | 0.005             |
| 9   | 4.5      | 25       | 150      | 10       | 0.029             |

3.4 *H. coralloides* EPS characterization

EPS of *H. coralloides* was composed of chains with an average molecular weight (Mw) of 413 and 1578 Da, but the frequency of the 1578 Da chain was higher (Fig. 3a). Low molecular weight polysaccharides are often composed of glucose subunits. These polysaccharides can induce apoptosis in cancer cells by entering them as well as display high antioxidant activity [31, 32]. This suggestion was consistent with the results obtained further in this work.

The stretching peak at 3466 cm\(^{-1}\) indicated vibrations of OH groups from polysaccharide chains (Fig. 3b) [33, 34]. The peak at region 2900 cm\(^{-1}\) corresponded to stretching of CH from aldehyde group. The 1577 cm\(^{-1}\) peak indicates the C=C vibration bond in polysaccharides [33, 35]. Also the 1426 cm\(^{-1}\) is attributed to alkane. Absorption peak was recorded at around 1043 cm\(^{-1}\) which indicates β-glucan in polysaccharides [33], and vibration of C–O–C appeared at
1012 cm\(^{-1}\) too [36]. Also, the absorption bands at 924 cm\(^{-1}\) are ascribed to the \(\alpha\) - and \(\beta\)-type glycosidic linkages [37]. The peak around 700–800 and 600 cm\(^{-1}\) is attributed to mannan band and the C–H bending [38].

Examining the XRD spectrum is a suitable method for determining the structure of polysaccharides. As shown in Fig. 3c, the XRD spectrum of the polysaccharide of \(H.\) coralloides showed the only amorphous peak at 20°. An absence of sharp peak in the XRD pattern indicated the low-crystallinity amorphous structure of the EPS.

### 3.5 Biological assessment

#### 3.5.1 Antioxidant assay

Figure 4a shows that the antioxidant activity of \(H.\) coralloides EPS increased linearly in the range of concentrations of 1–10 mg/mL with EC\(_{50}\) of 6.59 mg/mL. At these experimental conditions, a scavenging capacity of the positive control (BHT, 2 mg/mL) reached 83%.

#### 3.5.2 Cytotoxicity assay

The cancer cells, treated with EPS for 24 or 48 h, were affected in a dose-dependent manner (Fig. 4b, c). EPS was significantly more toxic for the AGS cell line compared to MKN-45. After 24 h of the treatment with 1 mg/mL EPS, the viability of AGS and MKN-45 cells reached 50.01 and 57.27%, respectively. EPS cytotoxicity on cancel cells almost doubled after 48 h of the treatment. Thus, the cell viability of AGS and MKN-45 cells at 1 mg/mL of EPS dropped till 20 and 30%, respectively.

### 4 Discussion

In the present study, the first native to Iran species \(H.\) coralloides was isolated, identified, and registered in NCBI under access number MW136052. The results showed that the molecular code EU784262 was very similar to the sequence of the present fragment [39]. The fungus also has a close phylogenetic relationship with \(H.\) erinaceum [40]. To obtain maximal EPS yield in vitro, the physicochemical culture conditions were examined. The optimum rotation speed for growth and EPS production in most fungi is 150 or 200 rpm. It was reported earlier that rotation speed affects the production of intracellular polysaccharides and EPS [41]. The ideal \(H.\) coralloides morphology for producing EPS is pellet hypha. Studies show that maltose is a good carbon source for basidiomycetes which stimulates their growth. This sugar is also preferred for \(H.\) erinaceum to increases its EPS production [30]. Huang et al. (2007) optimized the culture composition of \(H.\) erinaceus CZ-2: corn flour (30 g/L), glucose (10 g/L), yeast extract (3 g/L), KH\(_2\)PO\(_4\) (1 g/L), CaCO\(_3\) (0.35 g/L), and soaked corn (15 ml/L) [29]. It has been observed that the use of several nitrogen sources can increase the production of biomass and EPS in basidiomycetes [32, 42]. Soy peptone and yeast extract also accelerate the growth and production of fungal metabolites in comparison with inorganic sources of nitrogen [42]. The optimal conditions included 50 g/L maltose, 3 g/L soy peptone, and 1 g/L yeast extract at pH = 6.5, 200 rpm, 5% v/v inoculation, and 22 °C. In these conditions, EPS production reached 0.13 g/L. To the best of our knowledge, there is no study on the optimization of EPS production of \(H.\) coralloides. However, the EPS production of \(H.\) erinaceus ranged from 0.68 to 1.3 g/L, depending on the culture medium. GPC analysis showed \(H.\) coralloides EPS composed of chains with 413 and 1578 Da average molecular weights. It seems that \(H.\) coralloides EPS has a similar structure to \(H.\) erinaceus EPS; studies showed that the molecular weights of \(H.\) erinaceus EPS are lower than 15 kDa [43–46]. Low-weight polysaccharides have good antioxidants and anti-proliferative activity, which was also observed in our study. Examination of the FTIR spectrum confirmed the polysaccharide structure of \(H.\) coralloides. Comparison of the obtained peaks shows that these polysaccharides consist of beta-glucan chains and have glycosidic linkages [33, 37]. The XRD pattern shows

### Table 2

| Run | Factor 1 | Factor 2 | Factor 3 | Response |
|-----|----------|----------|----------|----------|
| A: Maltose (g/L) | B: Yeast extract (g/L) | C: Soy peptone (g/L) | EPS (g/L) |
| 1   | 30       | 2        | 2        | 0.080    |
| 2   | 30       | 3.31     | 2        | 0.052    |
| 3   | 30       | 2        | 2        | 0.076    |
| 4   | 10       | 3        | 3        | 0.060    |
| 5   | 10       | 3        | 1        | 0.045    |
| 6   | 30       | 2        | 3.31     | 0.076    |
| 7   | 50       | 3        | 1        | 0.041    |
| 8   | 10       | 1        | 1        | 0.030    |
| 9   | 30       | 2        | 2        | 0.053    |
| 10  | 30       | 2        | 2        | 0.060    |
| 11  | 10       | 1        | 3        | 0.040    |
| 12  | 50       | 1        | 1        | 0.062    |
| 13  | 30       | 0.68     | 2        | 0.040    |
| 14  | 30       | 2        | 2        | 0.076    |
| 15  | 50       | 1        | 3        | 0.144    |
| 16  | 56.32    | 2        | 2        | 0.094    |
| 17  | 3.67     | 2        | 2        | 0.031    |
| 18  | 50       | 3        | 3        | 0.116    |
| 19  | 30       | 2        | 2        | 0.079    |
| 20  | 30       | 2        | 0.68     | 0.034    |
that *H. coralloides* polysaccharides have a low-crystallinity amorphous structure. The degree of crystallinity significantly affects polysaccharides’ physicochemical properties and biodegradation [47]. Also, high crystallinity in some pharmaceutical molecules causes their solubility to decrease [48]. Decreased solubility can affect the availability and, thus, the performance of polysaccharides. A similar structure of the polysaccharides was observed earlier using basidiomycete fungi such as *Ganoderma lucidum* and *Ganoderma leucocontextum* [36, 49]. Structural studies on Shiitake mushroom polysaccharides also showed that the polysaccharides of this mushroom were amorphous [48]. However, more studies are needed to investigate the effect of crystallinity on biological properties.

Biological activities of *H. coralloides* EPS were also examined. This EPS scavenged DPPH free radicals (EC<sub>50</sub> = 6.59 mg/mL). Preliminary studies showed a comparable antioxidant activity of the *H. coralloides* extracts. Thus, methanol extract of *H. coralloides*, tested in DPPH, FRAP, and ABTS assays showed EC<sub>50</sub> of 4.12, 17.0, and 2.83 mg/mL, respectively [25]. The ethanol extracts of *H. coralloides* and *H. erinaceum* had similar radical

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**Table 1**

| #  | Name     | $M_n$ | $M_w$ | $M_z$ | $M_w/M_n$ | $M_z/M_w$ |
|----|----------|-------|-------|-------|-----------|-----------|
| 1  | Sample T | 1480  | 1578  | 1676  | 1.06622   | 1.06229   |
| 2  | Sample T | 401   | 413   | 425   | 1.02965   | 1.02909   |

**Fig. 3** GPC analysis of *H. coralloides* EPS molecular weight (a), FTIR (b) and XRD (c) of *H. coralloides* EPS
scavenging abilities with EC$_{50}$ of 7.19 and 5.82 mg/mL, respectively [50]. The comparable data could point out the optimal culturing conditions, selected in our study. As published earlier, optimizing the culture medium can increase the antioxidant activity of fungal polysaccharides by more than twice. Alone with a type of solvent, the solvent to fermentation medium ration can also affect the antioxidant activity of the isolated EPS. As shown in the work of Huang et al. (2013), the antioxidant activities of EPS fractions were strongly affected by the ratio of ethanol to fermentation medium used during precipitation step. The ratio 2:1 showed moderate and 5:1 maximal antioxidant activity, respectively. To take this parameter into account, the ratio 4:1 was used in the present study. Three
new compounds, isolated from the culture broth of *Hericium coralloides*, exhibited antioxidant activity in ABTS radical-scavenging assay with IC\textsubscript{50} values of 29–66 μM [51].

*H. coralloides* EPS has anti-proliferative activities on cancer cell lines and decreased viability of the cancer AGS and MKN-45 cells more than 80 and 70%, respectively, after 48 h of the treatment. Earlier studies showed strong anti-cancer potential of the polysaccharides of the genus *Hericium* [52]. β-Glucans can bind to specific receptors to activate macrophages, dendritic cells, neutrophils, and monocytes, and stimulate humoral and cellular immunity. As a result, by launching various signaling cascades, in addition to strengthening the immune system, they also stimulate it to fight cancer [53–55]. The anti-cancer activity of *H. erinaceus* polysaccharides was shown on human liver cancer (Hep G2), human breast cancer (MCF-7), and colon cancer (HCT 116) cells [56]. These polysaccharides can cause cell cycle arrest in the S-phase or induce apoptosis, modulating the caspase-8/-3-dependent, p53-dependent, mitochondrial-mediated, and PI3k/Akt-dependent signaling in cancer cells [57, 58]. Aqueous extract of *H. erinaceus* reduced the formation of tumor nodules in the lungs almost twice [59]. Studies showed that *H. erinaceus* polysaccharides prevented migration of the cancer cells [52]. Our results as well as already-published data indicated that *Hericium* polysaccharides have strong antitumor activity. We suggested that a new isolated native to Iran *H. coralloides* species can be considered in the future as a dietary supplement for cancer treatment. Further studies are necessary to understand the mechanism of the *H. coralloides* EPS activity on the cell cycle of cancer cells and to prove its action in vivo. Also, the low weight of *H. coralloides* EPS allows it to be loaded into drug delivery nanosystems in combination with anti-cancer drugs and to prove its action in vivo. Also, the low weight of *H. coralloides* EPS activity on the analysis of intracellular metabolites and cellular polysaccharides on the analysis of intracellular metabolites and the putative mechanisms of action. Fungal Divers 55(1):1–35

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