Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Nutritional and mineral contents of *Inocutis levis* and antioxidant activity of its fermented mycelia

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ARTICLE INFO

Article History:
Received: 2023/12/24
Accepted:2024/6/1

Keywords:

polypore fungi,

mycelium cultivation,

antioxidant,

nutritional value.

DOI: 10.22034/FSCT.21.154.78.

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ABSTRACT

The Elm polypore mushroom *Inocutis levis* is a macroscopic basidiomycete belonging to the Hymenochaetaceae family. Many members of this family have been of interest to researchers due to their medicinal and nutritional properties. Recent investigations have shown some bioactive properties of the fruiting body of *I. levis*. In this research, the nutritional and mineral composition of *I. levis* and the antioxidant activity of its mycelial extract have been investigated for the first time. Nutritional composition was determined according to AOAC method and mineral content was determined using ICP-MS. Mycelium cultivation was performed in PDA and then in PDB medium. Antioxidant activity of the fruiting body and mycelial extracts was evaluated via ABTS and DPPH assays. According to the results, *Inocutis levis* contains 14.2% protein, 73.7% carbohydrates (including 59.7% fiber), and a significant amount of minerals (11.6%) including potassium, phosphorus, magnesium, and iron. The concentration of lead and cadmium in *I. levis* is at the safe level, but the Health Risk Index (HRI) values of mercury and arsenic exceed the safe level. Although *I. levis* fruiting body extracts had a higher free radicals inhibitor activity, due to the significant yield of the mycelium extract and also the ease of cultivation, the cultured mycelium of this mushroom can be considered as a potential source of bioactive compounds. To apply this mushroom in food and

[DOI: 10.22034/FSCT.21.154.78]

pharmaceutical industries, a deeper investigation of the compounds of *I. levis* is suggested.

1- Introduction

Mushrooms have long been in the service of mankind not only as a delicious food but also as a nutritious and medicinal natural product. Edible mushrooms are used as food products worldwide and are often considered a suitable substitute for meat because they are rich in protein, crude fiber, vitamins, carbohydrates and minerals, but have low fat content [1]. Polypores are a group of fungi that form macroscopic fruiting bodies containing pores or tubes in the lower part and are a morphological group belonging to the phylum Basidiomycota [2]. These mushrooms have been used by human societies for a long time in various ways as food, nutritional supplement, tea, fire starter, medicine, etc. [3]. Among the most characteristic polypores with medicinal properties are various *Ganoderma* species as well as Chaga (*Inonotus obliquus*). The species studied in this research is Elm polypore with the scientific name *Inocutis levis* (P. Karst.) Y.C. Dai, belonging to the Hymenochaetaceae family. Several members of this family, especially species in the genera *Inonotus* and *Phellinus*, have been studied for their significant medicinal properties, and a number of species have a long history of food and medicinal use in Japan, Korea, China, and India. The genus *Inonotus*, with more than 100 species, is polyphyletic, and DNA analyzes have led to its division into more homogeneous taxa such as *Inocutis* [4, 5]. *Inocutis levis* is distributed from Central Asia to North Africa [6] and mainly grows on trees in urban areas. It is also widely distributed in Iran [7].

In 2005, Vinogradov et al. identified the structure of a polysaccharide isolated from *I. levis* [8]. In 2017, Ehsani Fard et al. found that the aqueous extract of *I. levis* increased insulin resistance and glucose tolerance in sucrose-fed Wistar rats [7]; they also showed in 2019 that in mice treated with aqueous extract of *I. levis*, the level of triglycerides in the blood serum decreased and in addition, the aqueous extract of this mushroom reduced leukocyte infiltration in the liver tissue [9]. In 2020 and 2024, Chaharmiri-Dokhaharani et al. analyzed a number of phenolic compounds of this species and showed antioxidant, antibacterial and non-cytotoxic properties in the fruiting body of *I. levis* [10 and 31]. Recently, during the research conducted by Chaharmiri-Dokhaharani et al. [31] and the authors of this article on *I. levis*, the presence of fatty acids such as oleic, stearic acid, linoleic, and hydroxyoctadecadienoic acids, and steroid compounds such as ergosterol, ergosterol peroxide, demethylincisterol A3, and cerevisterol have been determined.

Despite the existing evidence on medicinal aspects and bioactive compounds in *Inocutis levis*, the nutritional aspects of this mushroom are still unclear. In this study, for the first time, we investigate the nutritional and mineral characteristics of *I. levis*. In addition, by comparing the antioxidant property of the fruiting body and mycelium, it will be possible to optimize and more effectively use the biological properties of this macroscopic fungus. Therefore, this research aims to investigate the antioxidant properties of cultured mycelia in *I. levis*.

2-Material and methods

[DOI: 10.22034/FSCT.21.154.78]

2-1- Fungal sample

Inocutis levis was collected from trees in urban areas in Tehran in 2021. The identification of the sample and examination of its microscopic and macroscopic characteristics were done by Ghobad-Nejhad [11]. After preparing the mycelium culture, the dry mushroom samples were stored in the Iranian Cryptogamic Herbarium with the international index ICH.

2-2- DNA extraction and identification

In order to confirm the identity of the fungal mycelium, its genomic DNA was extracted with extraction kit and the ITS marker region [12] was amplified with ITS1F and ITS4 primer pair [13]. After amplification (Thermo Hybaid thermocycler) and horizontal electrophoresis, sample sequencing was done through Pishgam company. Finally, sequence confirmation and sample identification were performed by comparing with NCBI BLAST database sequences. The fungal isolate with the accession number IRAN 4562C was registered in the national collection of living fungi of Iran with the international index IRAN, and the obtained sequence was registered with the number OR978377 in the GenBank.

2-3- Mycelium cultivation

Cultivation of *Inocutis levis* was carried out on PDA (Potato Dextrose Agar) and then transferred to PDB (Potato Dextrose Broth) liquid medium. After incubating the culture at 25°C for 7 days on a rotary shaker (200 rpm), mycelial biomass was obtained. In order to prevent the growth of bacteria, tetracycline antibiotic with a final concentration of 10 mg/liter was used in the culture medium.

2-4- Examining the composition of nutrients

The analyses of moisture content, crude protein and fat, fiber, ash, and carbohydrate content were done according to the AOAC (1995) method [14]. The moisture content was determined by heating the sample at 105 °C until constant weight was reached. The amount of crude protein was estimated by the Kjeldahl method and the amount of total fat was estimated using a Soxhlet extractor and using petroleum ether as a solvent. After defatting, concentrated sulfuric acid and sodium hydroxide were used to estimate crude fiber. To determine the amount of ash, the sample was placed in a furnace with a temperature of 600 ± 15°C. The following formulas were used to calculate total carbohydrates and energy [15]:

Total carbohydrates % = 100 – (moisture + protein + fat + ash) %

Total energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × g fat

2-5- Examination of mineral content

The amount of elements sodium (Na), potassium (K), iron (Fe), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), molybdenum (Mo), selenium (Se), lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) was analyzed in *I. levis* with an Inductively Coupled Plasma-Mass Spectrometer (Agilent Technologies Inc. - 7500 ICP- MS, USA). In order to prepare the sample, 5 ml of HNO3 was added to 0.1 g of mushroom powder. By indirect heating, the sample was completely dissolved in acid, and the filtered solution with pure water reached a volume of 15 ml, and the dilution factor was included in the final result. ICP-MS measurements were performed using the following device conditions: 1200 W radio frequency generator power with 24 MHz resonance frequency, argon as nebulizer and auxiliary gas with a flow rate of 0.8 L/min., argon plasma flow rate of 12.2 L/min., 260 seconds sample absorption time, CCD solid state detector and a cyclonic modified Lichte spray

[DOI: 10.22034/FSCT.21.154.78]

chamber. Internal standards IV-STOCK-8 and IV-STOCK-24 (Merck, Germany) were used for calibration and quality control. To calculate the daily intake (DI) of heavy metals and their health risk index (HRI), the following formulas were used [17, 16 and 18]:

DI = mean concentration of toxic element in mushroom (mg/kg dw) × daily mushroom consumption (g/day)/Average body weight (kg)

HRI = DI/oral reference dose (RfDo)

In our study, the average body weight of a person is 70 kg and the daily consumption of mushrooms is considered to be 30 grams dried mushroom. The oral reference amount indicates the amount of exposure to oral pollutants during life and is 0.5, 0.3, 0.3 and 4 µg/kg/day for Cd, Hg, As and Pb elements, respectively [17, 16, 18].

2-6- Extraction

Fruiting body and dried mycelia of *I. levis* were powdered by electric mill (AIDISH, Iran). Extraction was done with 80% methanol and pure acetone solvents by soaking method. A mixture of 10 g mushroom powder in 100 ml of solvent was prepared and extraction was done for 24 hours at room temperature with the aid of a shaker (Kuhner SW) at a speed of 200 rpm. The resulting extract was filtered with Whatman filter paper and dried at 45°C with a rotary machine (APV Anhydro) and its solvent was evaporated. Extraction was repeated two more times on the remaining mushroom powder, and the efficiency was obtained from the following equation:

Yield % = amount of extract in grams / amount of mushroom powder in grams × 100

2-7- Examining the antioxidant property

In order to compare the antioxidant properties of the fruiting body and mycelium extracts of *I. levis*, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were used. The ABTS solution (7 mM) was prepared in 2.45 mM potassium persulfate ($K_2S_2O_8$) and incubated for 16 hours at room temperature and in the dark. Then, it was mixed with PBS (Phosphate-buffered saline) and its absorbance at 734 nm wavelength reached 0.7 ± 0.02 [19]. Twenty microliters of mushroom extracts with final concentrations (0.1, 0.5, 1.0, 1.5 and 3.0 mg/ml) were mixed with 980 microliters ABTS solution and the absorbance of the samples was read after 6 minutes at 734 nm. PBS solution was used as a control sample. In the DPPH method, 7.88 mg of DPPH powder was dissolved in 100 ml of pure methanol. Ten microliters of mushroom extracts with final concentrations of 0.5, 1.0, 2.5, 5.0 and 10.0 mg/ml were mixed with 190 microliters of DPPH solution and their absorption was read after 30 minutes at 517 nm wavelength [20 and 21]. Methanol was used as a control sample. In both methods, the radical inhibition percentage was calculated with the following equation. Also, IC_{50} (concentration of the sample capable of scavenging 50% of radicals) was obtained using linear regression. [Trolox was used as a positive control in concentrations of 5, 10, 15, 20, 25, and 30 µg/ml, and the inhibition percentage graph was drawn for it.].

Radical inhibition % = (control absorbance – sample absorbance) / control absorbance × 100

3-Results and discussion

3-1- Composition of nutrients

Proximate analysis results for *I. levis* mushroom show that this mushroom contains 90% moisture, 14.2% protein, 0.5% fat, 11.6% ash, 73.7% carbohydrates (including 59.7% fiber) and about 356 kcal energy per 100 grams of dry sample. According to the results (Figure 1), fiber (59.7%) constitutes the highest

[DOI: 10.22034/FSCT.21.154.78]

nutrient content of this mushroom. In mushrooms, the total carbohydrate content includes both digestible and non-digestible carbohydrates. Non-digestible carbohydrates include beta-glucans, mannans and non-starch polysaccharides, which are useful fibers for the diet [22]. In 2019, Ulziijargal et al. investigated the nutritional composition of the fruiting body and mycelium of different fungal species including *Inonotus obliquus*. This species contained 1.5% protein, 2.3% fat, 8.36% ash and 87.7% carbohydrates

[DOI: 10.22034/FSCT.21.154.78]

in its fruiting body, including 34.5% fiber in dry weight [23]. The composition of nutrients in the mycelium of *I. obliquus* was 25% protein, 5.7% fat, 3.3% ash and 65.4% carbohydrate (including 4% fiber). Since not much research has been done on the genus *Inocutis* especially in the field of food science, it is not possible to effectively compare the nutritional content of *Inocutis levis* with its counterparts. But compared to *I. obliquus*, the fruiting body of *I. levis* has significantly more protein and fiber. Although *I. levis* mushroom is inedible due to its hard tissues, this study suggests further investigation of its polysaccharides as a food additive or supplement. For example, recently Zhou et al. used a new polysaccharide extracted from *I. obliquus* in fermented yogurt and showed that this polysaccharide has potential application as a natural food additive in yogurt development due to its health benefits [24]. Also, the present study suggests further investigation of *I. levis* for the development of new food products. For example, the stipe of the fruiting body of shiitake mushroom (*Lentinula edodes*) is usually discarded because of its hard texture; however, Lin et al. (2010) suggested that they can be used as an alternative nitrogen source in alcoholic fermentation because they have a high protein content [25]. In 2009, *Ganoderma lucidum* was used by Yang et al. to ferment soy milk. The soy milk after fermentation by this fungus showed better acceptability and increased health properties [26, 27].

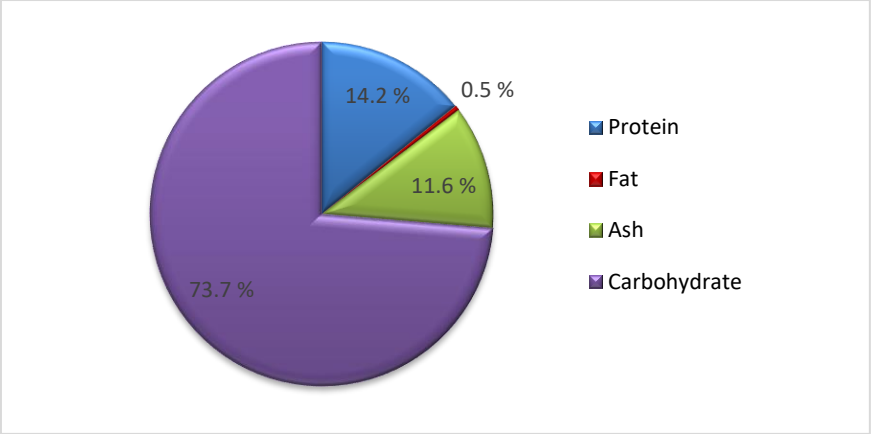

Fig. 1. Nutritional composition in *Inocutis levis* (100 g dry wight).

3-2- Mineral content

The concentration of mineral elements in the fruiting body of *I. levis* is shown in Tables 1 and 2. According to the results, the order of mineral content is K > P > Mg > Zn > Na > Ca > Fe > Cu > Mn > Se > Cr > Mo and the order of heavy metals is As > Hg > Cd ≥ Pb. Potassium is the most abundant element and sodium is the fifth element. Therefore, the Na/K ratio is less than 0.064. According to the recommendations of the World Health Organization (WHO), sodium intake should not exceed 2000 mg per day and potassium intake should not exceed 3510 mg per day. As a result, the Na/K ratio ≤1.0 is optimal for cardiovascular health [28]. The mineral content of *I. levis*, assuming that each person consumes 30 g of dried mushrooms per day, compared to the Daily Values (DVs) reported by the FDA in March 2020 is shown in Table 1. According to the results, *I. levis* can be a good source of minerals, especially potassium, phosphorus and magnesium. The updated FDA daily values for Cu and Cr have been decreased compared to the initial values. If we compare the amounts of copper and chromium in the mushroom with the initial amounts (2.00 and 0.12 mg, respectively), the daily consumption of 30

[DOI: 10.22034/FSCT.21.154.78]

grams of dried *I. levis* mushroom will be harmless for humans, but according to the updated values, both the content of copper and Cr will exceed the recommended limit. Also, the content of zinc and selenium in 30 grams of dried *I. levis* is significantly higher than the daily amount recommended by FDA (initial and updated values).

In this research, the Health Risk Index (HRI) was calculated in order to evaluate heavy metals in the mushroom (Table 2). In general, an HRI higher than 1 for any metal in food indicates that the consuming population is at risk [29]. The values in Table 2 show that the concentration of lead and cadmium in the investigated mushroom is at a safe level, but for As and Hg, the HRI value exceeds the safe level, which means that the concentration of these elements in *I. levis* needs to be monitored. In general, the bioaccumulation of heavy metals by fungi is a complex process and is influenced by various environmental factors, and different species behave differently based on their physiological needs [15]. Since in the present study, mushrooms were collected from trees in the urban areas around Tehran, investigating the amount of As and Hg in the air where the mushroom grows or even its substrata seems necessary for a more comprehensive understanding of the bioaccumulation of arsenic and mercury in *I Levis*. Recently, there is a growing public interest in harvesting wild edible mushrooms from urban and suburban environments. However, several studies have shown that mushrooms can contain high amounts of heavy metals or potentially harmful elements (PHEs), especially when they are collected from polluted and industrial areas [30]. Therefore, their consumption can be dangerous for humans, and the direct consumption of mushrooms harvested from urban areas requires deeper investigations.

Table 1. Mineral contents in *Inocutis levis* (mg/kg dry weight). FDA DVs, daily values reported by Food and Drug Administration.

Elements	**Concentration in *Inocutis levis***	**FDA DVs (mg)**	***Inocutis levis* DVs (for 30 g of dw)**
Na	645.61	2300	19.36
K	>10000	4700	>300
Fe	112.55	18.0	3.37
Ca	521.12	1300	15.63
P	4628.25	1250	138.84
Mg	3025.90	420	90.77
Mn	27.92	2.3	0.83
Cu	51.60	0.9	1.54
Zn	857.78	11	25.73
Cr	3.90	0.03	0.11
Mo	1.31	0.045	0.03
Se	9.73	0.055	0.29

Table 2. Heavy metal contents in *Inocutis levis* (mg/kg dry weight).

Elements	**Concentration in *Inocutis levis***	**Daily Intake (DI)**	**Health Risk Index (HRI)**
Pb	<0.1	<0.04	<0.01
Cd	<0.1	<0.04	<0.08
As	2.41	1.03	3.43

[DOI: 10.22034/FSCT.21.154.78]

Hg	1.85	0.79	2.63

3-3- Extraction efficiency

Extraction efficiency values from the fruiting body and mycelia of *Inocutis levis* are shown in Figure 2. In general, the amount of extract obtained with 80% methanol solvent is more than that of acetone. It is noteworthy that the yield of extraction with 80% methanol from *I. levis* mycelium is twice the yield of extraction from its fruiting body (50.2% and 24.2%, respectively). But extraction efficiency with acetone solvent is not much different in mycelia and fruiting body (3.5% and 4.8%, respectively).

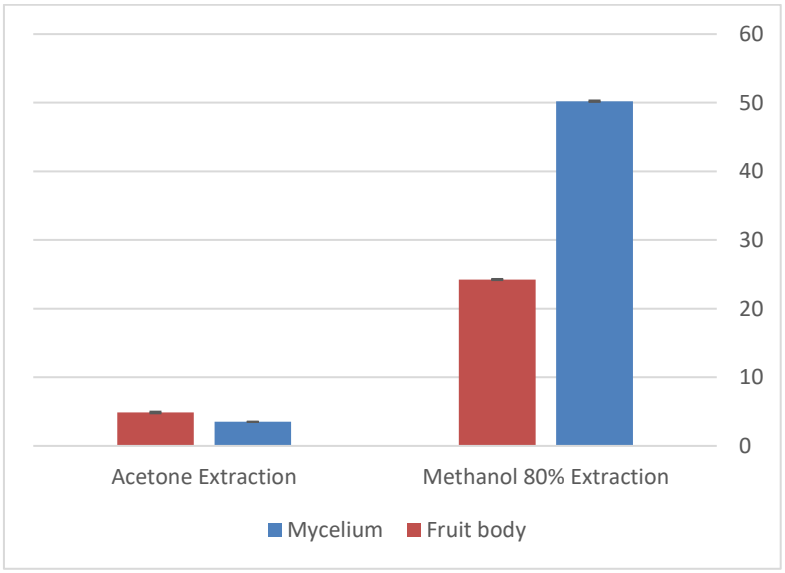

Fig. 2. Comparison of extraction yields in fruiting body and mycelia of *Inocutis levis*.

3-4- Antioxidant property

The results of investigating the antioxidant properties of *I. levis* are shown in Table 3. In general, methanol extracts (80%) had higher antioxidant properties than acetone (IC_{50} values equal to 0.3 and 0.8 mg/ml by ABTS assay, respectively). The highest free radical inhibition was related to the methanolic extract (80%) of the fruiting body. In both ABTS and DPPH methods, methanolic and acetone extracts of the fruiting body have lower IC_{50} and, as a result, higher antioxidant properties than mycelium extracts. Chaharmiri-Dokhaharani et al. reported an IC_{50} equal to 0.35 mg/ml for the acetone extract of the fruiting body of *I. levis* and an IC_{50} equal to 0.55 mg/ml for its methanolic extract (100%) by DPPH method [31].

The present study investigated the antioxidant property of *Inocutis levis* mycelia for the first time. Although the fruiting body extracts of *I. levis* had a higher power in inhibiting free radicals, but due to the higher yield of mycelium extract, it can be considered as a source of bioactive substances with antioxidant properties. Different studies show that in addition to the fruiting body, the cultivated mycelia of the mushroom have also been studied as a potential source of bioactive compounds. For example, Angelini et al. (2019), investigated the inhibition of free radicals by the methanolic extract of *Inonotus hispidus* via DPPH, and showed that the inhibition

[DOI: 10.22034/FSCT.21.154.78]

percentage was about 17.2 and 22.1% of Trolox activity in the fruiting body and mycelia, respectively [32]. Cultivated mycelia are becoming a promising alternative as a source of bioactive fungal compounds due to shorter incubation time and easier cultivation conditions (less space required, less contamination and higher biomass production) compared to fruiting bodies [33].

Table 3. Antioxidant properties in fruiting body and mycelia of *Inocutis levis* expressed as IC_{50} values (mg/ml).

Antioxidant activity of *Inocutis levis*	Acetone extract of mycelium	Acetone extract of fruiting body	Methanol extract of mycelium	Methanol extract of fruiting body
ABTS assay	4 ± 0.01	1.57 ± 0.01	0.86 ± 0.002	0.32 ± 0.001
DPPH assay	11.21 ± 0.02	7.6 ± 0.03	7.3 ± 0.01	1.09 ± 0.003

4- Conclusions

The use of natural resources such as wild plants for nutritional purposes requires the investigation of their chemical compounds and various biological properties [36–34]. The current study investigated the nutritional and mineral characteristics of the native macroscopic mushroom *Inocutis levis*. According to the results, this fungus contains 14.2% protein, 0.5% fat, 11.6% ash, and 73.7% carbohydrate (including 59.7% fiber). As a result, fiber (59.7%) constitutes the highest nutrient content of this mushroom. Also, *I. levis* has a high ash content, which is related to the high presence of macro and micro elements such as potassium, phosphorus, magnesium, and iron. All minerals examined in this study were at safe levels, except for zinc, selenium, mercury and arsenic, which were higher than recommended levels and may be hazardous to humans. Investigating the antioxidant properties of *I. levis* showed that the highest free radical inhibition was related to the methanolic extract (80%) of the fruiting body. In general, *I. levis* fruiting body extracts had a higher power in inhibiting free radicals, but due to the higher yield, the mycelial methanolic extract can be considered as a potential source of bioactive substances with antioxidant properties. The yield of methanolic extraction of mycelia was 50.2%, which means that more than half of the mycelium of this mushroom consists of different compounds that can have different biological properties, such as antioxidants. Therefore, the present study suggests further investigation of bioactive compounds and nutritional value of *I. levis* mycelium.

5- Acknowledgements

This article was prepared with the support of Iran National Science Foundation (No. 4000655). A previous grant from the International Association for Plant Taxonomy is acknowledged.

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[DOI: 10.22034/FSCT.21.154.78]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

محتوای غذایی و معدنی قارچ *Inocutis levis* و فعالیت آنتی‌اکسیدانی میسلیوم تخمیرشده آن

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۲/۱۰/۳

تاریخ پذیرش: ۱٤۰۳/۳/۱۲

کلمات کلیدی:

قارچ‌های پلی‌پور،

کشت میسلیوم،

آنتی اکسیدان،

ارزش غذایی

DOI:10.22034/FSCT.21.154.78.

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چکیده

قارچ پلی‌پور نارون با نام علمی *Inocutis levis* یک بازیدیومیست ماکروسکوپی متعلق به خانواده Hymenochaetaceae است. بسیاری از اعضای این خانواده به دلیل خواص دارویی و تغذیه‌ای مورد توجه محققان بوده‌اند. بررسی‌های اخیر، برخی خواص زیست‌فعال اندام بارده قارچ *I. levis* را نشان داده است. این تحقیق، برای اولین بار ویژگی‌های تغذیه‌ای و معدنی *Inocutis levis* و خاصیت آنتی‌اکسیدانی عصاره میسلیومی این قارچ را بررسی می‌کند. ترکیبات غذایی طبق روش AOAC و محتوای معدنی با استفاده از ICP-MS تعیین شد. کشت میسلیومی ابتدا در محیط کشت PDA و سپس در PDB صورت گرفت. مقایسه خاصیت آنتی‌اکسیدانی عصاره‌های اندام بارده و میسلیوم *I. levis* با سنجش‌های ABTS و DPPH انجام شد. بر اساس نتایج، این قارچ دارای ۱٤.۲ درصد پروتئین، ۷۳.۷ درصد کربوهیدرات (شامل ۵۹.۷ درصد فیبر) و همچنین مقادیر قابل‌توجهی از عناصر معدنی (۱۱.٦ درصد) مانند پتاسیم، فسفر، منیزیم و آهن است. غلظت سرب و کادمیوم در *I. levis* در سطح ایمن است، اما مقادیر جیوه و آرسنیک بر اساس شاخص خطر سلامت (HRI) از سطح ایمن فراتر می‌رود. نتایج نشان میدهد اگرچه عصاره‌های اندام بارده *I. levis* قدرت بالاتری در مهار رادیکال‌های آزاد دارد، اما به دلیل بازده قابل‌توجه عصاره میسلیومی و همچنین سهولت کشت، می‌توان میسلیوم کشت‌شده این قارچ را به‌عنوان منبعی بالقوه از ترکیبات فعال زیستی در نظر گرفت. برای استفاده از این گونه قارچی در صنایع غذایی و دارویی بررسی دقیق‌تر ترکیبات این قارچ پیشنهاد می‌گردد.

[DOI: 10.22034/FSCT.21.154.78]