Edible and Medicinal Mushroom *Hericium erinceus* - Potential Natural Material with Influence on Brain Functions

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Abstract

Hericium erinaceus (Bull.) Pers.,commonly known as lion's mane mushroom, monkey's head mushroom or Yamabushitake, is an edible medicinal mushroom with medicinal properties.

The aim of the study was to establish a mycelial cultures of H. erinaceus and use them for cultivation to obtain the fruiting bodies. Subsequently, the levels of indole compounds with antidepressant and procognitive activity were measured by RP-HPLC method in methanolic extracts from mycelium material and in extracts from fruiting bodies for comparison. The mycelium of H. erinaceus can be obtained in in vitro conditions. H. erinaceus is a rich source of indole compounds (5-hydroxy-L-tryptophan (5-HTP), melatonin and tryptamine). All of the investigated compounds were present in the H. erinaceus mycelium in higher quantity than in the mushroom's fruiting bodies. Interestingly, melatonin was only present in the mycelium but not in the fruiting bodies. The possibility of biotechnologically controlled cultivation opens an avenue for these medicinal mushrooms to be of use as a dietary supplement and even medicine in the future.

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Introduction

Currently, there is a lot of interest in functional food, which in addition to providing basic nutritional properties has a positive effect on human health. Mushrooms are now considered a functional food component, and their natural ability to accumulate various substances allows for the improvement of their health-promoting properties.

About 3,000 species of mushrooms have been recognized as edible, about a hundred of them are obtained commercially, but only ten species are obtained on an industrial scale. Mushrooms are valued for their taste and aroma and are widely used in folk medicine. Scientific publications from the last twenty years have proven many health-promoting properties of mushrooms. They exhibitanti-inflammatory, immunomodulating, antidiabetic, antioxidant, hepatoprotective, antitumor, anti-atherosclerotic, antiviral, antibacterial and antifungal properties. Mushrooms are a rich source of vitamins, minerals, phenolic and indole compounds, carotenoids, flavonoids, sterols and tocopherols [1, 2].

According to FAO data, the cultivation of mushrooms in the world amounted to over 10 million tons in 2016, so it is one of the fastest growing branches of horticulture. Over the past 10 years there has been a doubling of the harvest of fruiting bodies, as well as an increase in the species diversity of crops. A good example and explanation of this phenomenon can be the Japanese market, where the production of *Pleurotus eryngii* increased more than 5 times in less than 10 years, amounting to over 37 thousand tons in 2009. Currently, about 90% of mushroom companies operate in China, and the species most often grown on a commercial scale they belong to the genera *Lentinula*, *Pleurotus*, *Auricularia*, *Agaricus*, *Flammulina*, and they account for 22%, 19%, 18%, 15% and 11% of world production, respectively [3,4,5]

Hericium erinaceus (Bull.) Pers., commonly known as lion's mane mushroom, monkey's head mushroom or Yamabushitake, is an edible medicinal mushroom. It is widely found in East Asian countries (China, Japan), but also in Europe, where it is considered as an endangered species (Austria, Belgium, the Czech Republic, Switzerland, Germany, Denmark, England, the Netherlands, Sweden and Slovakia) In Poland *H. erinaceus* is under strict species protection.

Hericium erinaceus is cultivated in substitute substrate – sawdust of deciduous trees, grains bran and many other agriculture waste that is placed and sterilized in polypropylene bags or bottles. Commercial cultivation is increasing worldwide, especially in Asia country and USA, but still it is far less popular than white button mushrooms, oyster mushrooms and shiitake mushrooms [6].

Because of its possible nutritional and medicinal properties *H. erinaceus* was used in traditional medicine, but recently it has been found to promote positive nerve and brain outcomes as well. It is considered to have great potential in treating neurological disorders due to high content of neuroactive compounds that can pass through the blood–brain barrier. Bioactive compounds extracted from its fruiting body or mycelium have been demonstrated to possess antioxidative, antidiabetic, anticancer, anti-inflammatory and antimicrobial properties. Furthermore, *H. erinaceus* has been used as an adjunct treatment for cognitive impairments, Alzheimer's disease, Parkinson's disease and ischemic stroke. The present research on *H. erinaceus* focused on the biological underpinnings of its beneficial effects in affective disorders. Indole compounds in *H. erinaceus* seem to have the biggest potential to exert antidepressant effects.

The aim of the study was to establish a mycelial cultures of *H. erinaceus* and use them for cultivation to obtain the fruiting bodies. Subsequently, the levels of indole compounds with antidepressant and procognitive activity (5-hydroxy-L-tryptophan (5-HTP), melatonin and tryptamine) were measured by RP-HPLC method in the mycelium material and in methanolic extracts from fruiting bodies for

comparison.

Materials and method

Mushroom materials

Mycelial cultures

The mycelia of *H. erinaceus in vitro* cultures grown on the solid medium were transferred to 250 mL of the modified liquid Oddoux medium in order to obtain the highest possible amount of biomass for use in further analyses [7] The cultures were shaken at 140 rpm (Altel, Łódź) at 25±2°C under a cycle resembling natural light conditions.

To obtain efficient and significant growth of biomass, the mycelium from Erlenmeyer flasks was transferred to a biofermenter, in which the volume of the medium was 10 L; the cultures were mixed by the inflow of air passed through the sterile antibacterial filters. Carbon dioxide formed during the growth of the mycelium was constantly removed. The cultures were grown at a temperature of 25±2°C and under the natural photoperiod. After 10 days of growth in the biofermenter, the mycelium was separated from the medium and was frozen and lyophilized (Freezone 4.5 lyophilizer, Labconco; temperature:–40°C).The lyophilized samples were weighed and used for analysis.

H. erinaceus cultivation

Mycelia from the *in vitro* cultures were used for cultivation. The first stage of cultivation was to obtain grain mycelium. For this purpose, moistened wheat grains were placed in polypropylene bags with a microfilter. The bags were sterilized at 121°C for 1 h, cooled down, and then inoculated with *in vitro* cultures. Next step was preparing cultivation substrate, containing homogeneous beech sawdust and wheat bran in a 5:1 ratios and 1% horticultural gypsum. The substrate was thoroughly mixed, moistened to a relative humidity of 65%, and placed in polypropylene bags with a microfilter. Then, 2 kg of the substrate was placed in the bags and sterilized. After cooling down, 3% of the previously prepared grain mycelium was passaged on the substrate, the mixture was stirred, and cultivation cubes were formed. The cubes were incubated at 24±1°C. After the substrate was completely overgrown, the upper part of the foil was removed and the cubes were placed in the cultivation chambers where a humidity of 96±3%, a temperature of 17±2°C, and a light intensity of 500 lux per 12 h a day. Fruiting bodies were harvested when they reached the maturity stage, before the mass spore production. Only the first-flush, homogeneous fruiting bodies showing typical appearance were used, and the nonfood fragments were not intended for further analysis (Picture 1.).

Picture 1. *H. erinaceus* cultivation (Piotr Zięba)

After selection, the fruiting bodies were frozen, lyophilized, and ground to be used for chemical analyses.

Indole compounds analysis by RP-HPLC analysis

In order to assess the ability of the species *in vitro* cultures to produce indole compounds, an extraction process was performed with methanol. The obtained mycelium mushroom material and the fruiting bodies of *H. erinaceus* were extracted with methanol in an ultrasonic bath for 30 minutes. After the specified time had elapsed, the freeze-dried mycelium was separated from the extract using a filter paper. The sample was flooded with more methanol and placed in the bath to obtain a high-yield extraction process. The entire process was carried out a total of 4 times. The resulting filtrate was allowed to evaporate at room temperature, thus obtaining dry extracts. The dry extracts were washed with a sufficient amount of HPLC grade methanol and filtered through syringe filters [Millex, Millipore Corporation, USA]. Determination of indole compounds was performed according to the procedure described by Muszyńska [8]. Briefly, the conditions were as follows: Hitachi HPLC; pump L-7100; Purospher RP-18 column (250 mm x 4 mm, 5 μm). An isocratic separation was used, and the mobile phase was methanol: water: ammonium acetate 15: 14: 1 (v/v v); flow 1 mL / min. The chromatographic peaks were recorded at a wavelength of 280 nm. Indole standards were purchased from Sigma (St. Louis, USA).

Scavenging activity analysis (%DPPH·)

0.1 mL of mushrooms methanolic extracts made (50%) was mixed with 0.1 mM 2,2-Diphenyl-1-picrylhydrazyl(DPPH) (Sigma-Aldrich, St. Louis, USA) dissolved with 4.9 mL of 100% methanol. The mixture was shaken and kept in dark for 45 min. The absorbance was measured at 517 nm using a Ultraviolet-Visible Spectroscopy UV-VIS Helios Beta Spectrophotometer (Termo Fisher Scientifc Inc., Waltham, MA, USA). DPPH radical scavenging activity was calculated using the formula: AA (%)=[(A0−A1)/A0]×100, where AA is the antioxidant activity,A0 is the absorbance of the reference solution, and A1 is the absorbance of the test solution.

Total phenol content

Total phenolic content was estimated using the modified Folin-Ciocalteu colorimetric method. Additionally, 0.1 mL of mushrooms' methanolic extracts was mixed with 2 mL of sodium carbonate. After the next 2 min, 0.1 mL of Folin-Ciocalteu's reagent (Sigma-Aldrich, St. Louis, USA), mixed with deionised water (1:1v/v), was added. The absorbance of the resulting blue colour was measured at 750 nm using the UV-VIS Helios Beta spectrophotometer against a reference solution. The results are expressed as gallic acide quivalents (GAE).

Results and discussion

The mycelium of *H. erinaceus* can be obtained in *in vitro* conditions. *H. erinaceus* is a rich source of indole compounds (5-hydroxy-L-tryptophan (5-HTP), melatonin and tryptamine) and has high content of phenols (Table 1 and 2.).

Non-hallucinogenic indole derivatives (determined in fruiting bodies and mycelial cultures of H. erinaceus) with therapeutic importance include, among others serotonin, 5-hydroxy-L-tryptophan and tryptamine. These compounds fulfill the role of neurotransmitters or their precursors. They impact the daily cycle, regulating appetite, mood, and bodily temperature. They are responsible for coagulation processes, immune response, and cellular regeneration. Moreover, they demonstrate antioxidative (thus reducing lipid peroxidation), anticancer, cardioprotective and analgesic properties All of the investigated compounds were present in the *H. erinaceus* mycelium in higher quantity than in the mushroom's fruiting bodies.

Interestingly, melatonin was only present

Table 1. Indole compounds content in fruiting bodies and mycelium of *H. erinaceus*

Indole compound	*H. erinaceus* mycelium [mg/100 g d.w.]	*H. erinaceus* fruting bodies [mg/100 g d.w.]
Melatonin	1.04±0.03	*
Tryptamine	11.88±0.44	1.19±0.03
5-Hydroxy-L-tryptophan	152.72±10.79	92.19±3.36

*– below detection

Table 2. Total phenol content and Scavenging Activity (%DPPH·)

	H. erinaceus mycelium	*H. erinaceus* fruting bodies
Total Phenol content [mg/100 g d.w] mean±SD, GAE	560,98±4,82	206,55±8,65
DPPH·[%]	61,49±0,54	12,59±0,57

in the mycelium but not in the fruiting bodies (Table 1).

Melatonin (N-acetyl-5-methoxytryptamine) is primarily produced in pineal gland pinealocytes, as well as in the eye retina and in the gastrointestinal tract.

This compound participates in the regulation of sleep, mood and reproduction-it coordinates the operation of the biological clock controlling the circadian rhythm and has anti-ageing effect, regenerating processes of cellular regeneration. Moreover, it has anticancer effect. The biological effects are caused by the activation of melatonin receptors. Melatonin is available as a medicinal product, intended for supporting use in sleep disorders associated with change of time zones or shift work [8-10]. Moreover, melatonin is a strong antioxidant, delaying ageing processes neutralizing free radicals, such as the hydroxyl radical. Thus, melatonin can reduce damage caused by certain types of the Parkinson's disease. Moreover, research has proven its efficiency in treating Alzheimer's disease. Melatonin has neuroprotective role in neurodegenerative diseases [11-13]. Moreover, immunomodulatory and anti-inflammatory properties of melatonin have been demonstrated. Unfortunately, the level of melatonin decreases after 35 year, and considerably after 40 year of age, resulting in rapid human organism ageing and increased risk of neoplastic diseases. Mycelium from *in vitro* culture has two times more phenol content and four time more scavenging activity, compare to fruiting bodies. Phenolic compounds have strong antioxidants activity – consumption of phenolic-rich foods prevent cancer, type-II diabetes mellitus or cardiovascular disease (CVD) [14]. Much stronger scavenging activity (DPPH·) of mycelium obtain from *in vitro* culture suggest that other antioxidants compounds like diterpenes, steroids and other active metabolites can be found in mycelium with higher content than in fruiting

bodies. More research are need to verify it and compare content of different substance that can be found in mushrooms mycelium and fruiting bodies

Conclusions

The mycelium of *Hericium erinaceus* can be obtained in *in vitro* conditions. *Hericium erinaceus* is a rich source of indole and phenolic compounds. All of the investigated compounds were present in the *Hericium erinaceus* mycelium in higher quantity than in the mushroom's fruiting bodies. Interestingly, melatonin was only present in the mycelium but not in the fruiting bodies. The possibility of biotechnologically controlled cultivation opens an avenue for these medicinal mushrooms to be of use as a dietary supplement and even medicine in the future.

Resumo

Hericium erinaceus (Bull.) Pers., ofte konata kiel fungo de leona maneo, kapo de simio aŭ Yamabushitake, estas manĝebla funga medikamento enhavanta resanigajn propraĵojn.

La celo de esplorado estis establi miceliajn kulturojn de H. erinaceus kaj kultivadane ilin uzi por akiri ĝiajn korpajn fungojn. Poste la niveloj de indolaj komponaĵoj kun antidepresiva kaj nootropa efikoj estis mezuritaj per RP-HPLC metodo en metanolaj eltiraĵoj el micelia materialo kaj el korpaj fungaj eltiraĵoj. H. erinaceus mycelium estas akirebla in vitro.

H. erinaceus estas riĉa fonto de indolaj komponaĵoj (5-hidroksi-L-triptofano (5-HTP), melatonino kaj triptamino). Ĉiuj ekzamenitaj komponaĵoj ĉeestis en la micelio de

H. erinaceus en pli granda kvanto ol en la fungaj korpoj de la fungo. Interese, melatonino ĉeestis nur en la micelio, sed ne en la fungaj korpoj. La eblo de bioteknologie kontrolita kultivado malfermas la vojon por ke ĉi tiuj kuracaj fungoj estu uzataj estonte kiel dieta suplemento kaj eĉ kiel medikamento

References

1. Sułkowska-Ziaja K., Kała K., Lazur J., Muszyńska B. Chemical and Bioactive Profiling of Wild Edible Mushrooms. P. Singh et al. (eds.), Biology of Macrofungi, Fungal Biology, Springer Nature Switzerland AG 2018 129B. 129-157.

2. Bonneville, S., Delpomdor, F., Préat, A., Chevalier, C., Araki, T., Kazemian, M., Steele, A., Schreiber, A., Wirth, R., & Benning, L. G. (2020). Molecular identification of fungi microfossils in a Neoproterozoic shale rock. Science Advances, 6(4), 1-11. https://doi.org/10.1126/sciadv.aax7599

3. Bruns, T. (200). A kingdom evised. Nature, 443(7113), 758-761. https://doi.org/10.1038/443758a

4. Kayzer, D. (2017). Stan oraz perspektywy rozwoju branży pieczarkarskiej w Polsce The state and development prospects of the mushroom industry in Poland. Zeszyty Studenckie Wydziału Ekonomicznego Uniwersytetu Gdańskiego „Nasze Studia", (8), 230-238.

5. Muszyńska B., Kała K., Sułkowska-Ziaja. Mushrooms as a source of biological active indole compounds (chapter 2). Indole Synthesis, Functions and Reactions. Series: Chemistry Research and Aplications. Nova, ISBN 1536147773. January 2019.

6. Chang, S.T., Miles, P.G. (2004). Mushrooms. Cultivation, nutritional value, medicinal effect, and environmental impact. Boca Raton: CRC Press LLC.

7. Oddoux, L. (1957). Recherches sur les mycéliums secondaires des Homobasidiés en culture pure. Imprimerie de Trevoux, Lyon

8. Muszyńska B, Sułkowska-Ziaja K, Ekiert H (2011) Indole compounds in fruiting bodies of some edible Basidiomycota species. Food Chem 125:1306–1308 . doi: 10.1016/j.foodchem.2010.10.056].

9. Muszyńska B, Sułkowska-Ziaja K. "Impact of Food Processing on Non-Hallucinogenic Indole Derivatives in Edible Mushrooms". Processing and impact on active components in food. Elsevier, Amsterdam 2015; 55-62, ISBN 978-0-12-404699-3.

10. Singhal, N. K., Srivastava, G., Agrawal, S., Jain, S. K. and Singh, M. P. (2012). Melatonin as a neuroprotective agent in the rodent models of Parkinson's disease: is it all set to irrefutable clinical translation? Molecular Neurobiology, 45: 186-199.

11. Morgan, Y. R., Turner, P., Kennedy, B. J., Hambley, T. W., Lay, P. A., Biffin, J. R., Regtop, H. L. and Warwick, B. (2001). Preparation and characterization of dinuclear copper-indomethacin anti-inflammatory drugs. Inorganica Chimica Acta, 324: 150-161.

12. Paredes, S. D., Korkmaz, A., Manchester, L. C., Tan, D. X. and Reiter, R. J. (2009). Phytomelatonin: a review. Journal of Experimental Botany, 60: 57-69.

13. Reiter, R. J., Tan, D. X., Burkhardt, S. and Manchester L. C. (2001). Melatonin in Plants. Nutrition Reviews, 59: 286-290.

14. Wang, X. (2009). The anti-apoptotic activity of melatonin in neurodegenerative diseases. CNS Neuroscience & Therapeutics, 15: 345-357.

15. Shahidi, F., & Peng, H. (2018). Bioaccessibility and bioavailability of phenolic compounds. Journal of Food Bioactives, 4: 11-68.