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Role of mycelia derived from *in vitro* cultures of *Amanita* spp. as a potential source of bioactive compounds with therapeutic potential for the mitigation and management of depressive disorders

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Summary

Mushrooms of the *Amanita* genus are considered among the most toxic, causing severe poisoning, often resulting in death. However, of the 707 described species within this genus, only around a dozen contain the toxic octapeptides classified as amanitotoxins and phallotoxins. While most representatives of the genus are considered inedible species, there are a few exceptions that are palatable edible species. *Amanita muscaria* and *Amanita pantherina* fall into the category of poisonous species, with significant ethnomycological impact on human evolution and sociology, alongside their other psychoactive effects.

This study aimed to obtain mycelium of *A. muscaria* and *A. pantherina* species under controlled laboratory conditions, using 10 L air-lift bioreactors and to evaluate the obtained fungal material as a potential pharmaceutical raw material containing muscimol and other biologically active compounds of importance, which may have significance in the prevention of depression. The resulting biomass was analyzed by RP-HPLC and AAS to identify various organic compounds (indole compounds, sterols, lovastatin, ergothioneine, muscimol, and ibotenic acid) and different bioelements.

Based on the results obtained, it can be concluded that the mycelium of *A. muscaria* contains several bioactive compounds, such as lovastatin, ergothioneine, and 5-hydroxy-L-tryptophan, at higher levels than *A. pantherina*. The determination of muscimol and other bioactive substances, which have not been previously studied, in the biomass obtained through *in vitro* cultivation, compared to those found in the fruiting bodies, suggests the potential of these species in the

treatment of depression. However, further research, including *in vitro* experiments and subsequent clinical trials, is required.

Key words: indole compounds, muscimol, bioelements

1. Introduction

Fungi, the least-studied kingdom of *Eukaryota,* exert a significant impact on various aspects of human life. In their paper, Hyde et al. [1] described 50 opportunities that fungi offer to humans and presented fungal metabolites that could prove to be potential sources of medicines, valuable industrial and horticultural substances, and agents that can help remediate environmental pollution [2].

Fungi within the genus *Amanita* are considered among the most toxic organisms causing poisoning, often resulting in death. However, out of the 707 documented species, only a dozen contain highly toxic cyclic octapeptides from the amatoxin and phallotoxin groups, and most are classified as inedible. Interestingly, this genus includes some of the most poisonous as well as highly palatable edible species. *Amanita muscaria* and *Amanita pantherina* have left an indelible ethnomycological imprint on human evolution and society, primarily due to their psychoactive effects, among other factors. At the same time, some species, such as *Amanita rubescens*, are considered edible and this species is considered, by connoisseurs, to be a mushroom with a unique flavor and aroma. All *Amanita* species are mycorrhizal fungi, living with complex symbioses not only with specific tree species but also with bacteria and other fungi [3]. The genus *Amanita,* and notably its characteristic representative *A. muscaria*, stands as one of the most globally recognized fungal species, despite having been overlooked in scientific literature for a very long time. Several works describe the great cultural and historical significance of this species in ethnomycology, the study of the relationship between fungi and people, especially within cultural contexts [4, 5]. This species has been associated with humans since the very beginning of mankind, as evidenced by its use for mystical, ritual, and religious purposes, as well as in traditional medicine and shamanic practices [4, 5]. Scientific research into *Amanita* spp. began in the 20th century, but as the century drew to a close, the topic became less popular probably due to negative perceptions of and attitudes toward psychoactive substances. However, there has been a resurgence of interest in various shamanic practices, altered states of consciousness, and expanded perception by humans. This has resulted in increased consumption of psychoactive plants and mushrooms. This resurgence may be related to the increasingly fast pace of modern life, increased stress, and the consequent increase in the number of people suffering from depression and other central nervous system disorders. The complex topic of human use of *A. muscaria* has seen a growing interest in its potential for novel psychotherapeutic applications. In the United States of America, research is underway on the treatment of depression using psychoactive mushrooms designed to produce powerful psychedelic experiences [6, 7]. The effects of *A. muscaria* poisoning are dangerous for health, and recent years have seen an in-

crease in interest in its consumption. It should be noted that chronic consumption of its fruiting bodies or derived extracts can lead to death [8, 9].

Other important hallucinogenic mushrooms, known since prehistoric times, belong to the *Psilocybe* species [6, 10]. They were used primarily as part of religious and divination practices, but also for medicinal purposes. In Europe, these mushrooms were not more thoroughly understood until the second half of the 20th century. Psylocybin was isolated by Swiss scientist Albert Hofmann, who had also previously described the psychoactive properties of LSD (D-lysergic acid diethylamide) obtained from *Claviceps purpurea* (Fr.) Tul. Since then, mushrooms of the genus *Psilocybe* have been used primarily as tools to "expand consciousness," which has contributed to their abuse and, consequently, their classification as dangerous narcotic substances [6, 10, 11]. The late 20th and early 21st centuries observed a renewed interest in psilocybin and its derivatives. This time, its potential therapeutic value was described, particularly in the treatment of conditions such as depression, anxiety, post-traumatic stress disorder (PTSD) and substance abuse. The discovery of the potential health benefits of this type of hallucinogenic mushrooms has led to renewed discussion around the world about their legalization and possible approval for therapeutic use, taking into account the risks associated with psychedelic substances. Significantly, on July 1, 2023, Australia became the first country to officially authorize the use of psilocybin for the treatment of drug-resistant depression (information available on the Australian Government, Department of Health and Aged Care, Therapeutic Goods Administration website) [6, 11, 12]. The approval of such substances has also been important for other hallucinogenic mushrooms of potential therapeutic importance, including *Amanita* spp. [7, 12].

A. muscaria contains muscimol. The substance is an agonist of GABA receptors. These are membrane receptors that bind γ-aminobutyric acid in the human brain, causing psychoactive effects such as hyperactivity, hallucinations and delusions. Although muscimol acts on $GABA_A$ receptors in a similar manner as antidepressants do, it has not yet been used medically. Limited research was conducted in the 1970s for the clinical treatment of schizophrenia, but satisfactory results were not obtained [7,13].

Research is currently underway to utilize muscimol and determine the optimal dosage for its antidepressant effects. Importantly, muscimol is also present in other species of *Amanita* – it has been identified in *A. pantherina* [7, 14].

This study aimed to determine the content of compounds in the mycelia and fruiting bodies of *A. muscaria* and *A. pantherina* that may have potential use in the prevention and treatment of depression. The muscimol content in fresh *Amanita* spp. fruiting bodies is highly variable, dependent on individual factors, and can vary by as much as a few to several percent. This variability presents challenges in obtaining a good source of mushroom material from the wild [9]. The solution to this problem turns out to be the biotechnological obtaining of mycelium under laboratory conditions, which results in reduced environmental interference and reduced risk of inadvertently collecting the wrong species, while also providing opportunities for targeted production of specific bioactive substances.

The aim of the study was to obtain fruiting bodies from the natural environment and produce mycelia of two species – *A. muscaria* and *A. pantherina* – using biotechnological methods in 10 L bioreactors equipped with an airlift system, followed by the evaluation of the content of biologically active substances in the obtained material with particular emphasis on important compounds relevant to the prevention of depression. It should be emphasized that the objective of the study was not only to determine the levels of muscimol and ibotenic acid, but also, for the first time, to analyze the content of other substances of therapeutic importance, with special focus on non-hallucinogenic indole derivatives such as L-tryptophan, which is a precursor of serotonin synthesis in the central nervous system, and 5-hydroxy-L-tryptophan, which has proven antidepressant activity. Importantly, a comparative analysis of the content of both organic compounds and bioelements was conducted.

2. Materials

2.1. Research material

The fruiting bodies of *Amanita muscaria* (L.) Lam. and *Amanita pantherina* (DC.) Krombh were collected in mixed forests in southern Poland, near Kraków. Taxonomic identification was performed through mycological analysis (Prof. Bożena Muszyńska), referencing the key to fungal identification [15]. Specimens of the collected material are stored in the Department of Pharmaceutical Botany at the Jagiellonian University Medical College in Kraków (deposit numbers: KBF/AM—1/2022, KBF/AP—2/2022).

2.2. Production of mycelial cultures

Fresh fruiting bodies were used to initiate mycelial cultures. *In vitro* culture on an agar-solidified medium was carried out according to the procedure developed by Muszyńska et al. [16]. In the initial phase, stationary cultures were obtained on a modified medium based on the Oddoux methodology [17].

2.3. Shaken liquid cultures

To initiate liquid cultures, fragments of mycelium from agar cultures were transferred into 500 mL Erlenmeyer flasks, maintained through shaking (at a depth). These flasks were placed on a rotary shaker (ALTEL, Łódź, Poland) at a frequency of 140 rpm, operating at 25 ± 2 °C, under varying light conditions following a diurnal cycle. These cultures served as the source material for initiating experimental bioreactor cultures as part of the optimization process.

2.4. Experimental cultures in air-lift bioreactors

To obtain effective biomass growth for further analysis, mycelium from liquid shaken cultures was transferred to a 10 L bio fermenter (SIMAX® glass bottles). The biomass mixing was ensured through the supply of sterile air and CO_2 using an air-lift system. After a period of 10 days, the biomass was separated from the medium, frozen, and subsequently lyophilized. The resultant material was used in the subsequent phases of the study.

3. Methods

3.1. Extraction of the obtained material

The biomass (fruiting bodies and mycelia from *in vitro* cultures) was lyophilized in a lyophilizer (Labconco Freezone lyophilizer 4.5, Kansas City, USA) and then ground in an agate mortar. Samples of appropriate weights, 3 g each, were extracted for 20 min with methanol (100 mL) using 40 kHz ultrasound. This extraction process was repeated nine times for each sample. The extracts were centrifuged (15 min at 35,000 rpm). The resulting supernatant was then separated from the precipitate and placed in crystallizers to allow the methanol to evaporate at a temperature of 25 ± 2 °C. The resulting dry residue was quantitatively dissolved in HPLC-grade methanol (Honeywell Riedel-de Haën, Sheelze, Germany) and filtered using syringe filters (Millipore Millex® GP 0.22 µm, Merck, Darmstadt, Germany). The resulting extract was designated for HPLC analysis of the following compounds: muscimol, ibotenic acid, indole compounds, lovastatin, and ergothioneine.

3.2. Analysis of the content of indole compounds

For the determination of indole compounds, extracts obtained from fruiting bodies and mycelia from *in vitro* cultures were analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) with an HPLC analyzer (Merck Hitachi, Tokyo, Japan). The analytical setup included an L-7400 UV detector, L-2350 thermostat, L-7100 pump, VWR7614 degasser, and an RP-18 4 × 250 mm column (Purospher®, 5 µm). The column temperature was maintained at 25 °C. Isocratic separation was performed using a mixture of methanol, water, and ammonium acetate in a ratio of 15:14:1 v/v/v for the determination of L-tryptophan, melatonin, tryptamine, and 5-methyltryptamine. Detection was performed at λ = 280 nm. For the determination of 5-hydroxy-L-tryptophan, a mixture of 0.1% phosphoric acid and acetonitrile in a volume ratio of 93:7 was used as the eluent. The presence of this compound was analyzed at λ = 275 nm. The eluent flow rate for the analysis of all indole compounds was set at 1.0 mL/min and quantitative analysis was performed using the calibration curve method.

3.3. Analysis of ergothioneine content

Analysis of ergothioneine content was conducted through RP-HPLC in accordance with the method proposed by Zhou et al. [18], using the analyzer described above. Isocratic separation was achieved using a mixture of 1% methanol and boric acid (pH 5), with the column temperature set at 25 °C. Analysis was performed at λ = 275 nm, and the eluent flow rate was 0.5 mL/min.

3.4. Analysis of lovastatin content

To determine the lovastatin content in the samples, the method described by Pansuriya and Singhal [19] was used. The analysis was carried out by RP-HPLC using the same apparatus described earlier. Isocratic separation was performed using a 60:40 v/v mixture of acetonitrile and 0.1% phosphoric acid. The column temperature was set at 25 °C, and the wavelength used was λ = 238 nm. The eluent flow rate was 1.0 mL/min.

3.5. Analysis of muscimol and ibotenic acid content

The determination of muscimol and ibotenic acid was carried out according to the methodology developed by Tsunoda et al. [20,21], with modifications in the proportion of eluent used and analysis conditions. The liquid chromatography kit described earlier was used for these determinations. An isocratic elution was used with the eluent consisting of a mixture of water, acetonitrile, and methanol in a ratio of 60:20:10 v/v, with the addition of 4 mM phosphoric acid (pH 2.2), and sodium dodecyl sulfate (2.1 mM). The determinations were made at a wavelength of λ = 210 nm, and the column temperature was maintained at 35 °C.

3.6. Analysis of sterol content

For the analysis of sterols, 5 g of lyophilized fruiting bodies from *A. muscaria* and *A. pantherina* were weighed, and their mycelia were measured. These samples were extracted in a mixture of methanol and dichloromethane (75:25, v/v) in an ultrasonic bath operating at 40 kHz for 10 min. After 2 h, the resulting extract was centrifuged at 12,000 rpm for 5 min. This extraction process was repeated twice, and then the supernatant was separated from the precipitate and placed in 80 mL crystallizers allowing the methanol to evaporate. The resulting dry residue was dissolved in HPLC-grade methanol (Merck, Darmstadt, Germany) and filtered using syringe filters. These determinations were carried out according to the methodology outlined by Sułkowska-Ziaja et al. [22].

3.7. Analysis of β-glucan content

The content of β-glucans was determined using a test kit (Megazyme© International, Ireland Ltd, Wicklow, Ireland) as per the manufacturer's instructions and the procedure detailed by Sari et al. [23]. All samples of 0.1 g of lyophilized material were ground with an analytical grinder and sieved through a 0.5 mm mesh. In the next step, 1.5 mL of 37% HCl was added, and the mixture was heated at 30 °C for 45 min. Subsequently, 10 mL of distilled water was added to each sample, which was then incubated for 2 h in a boiling water bath. After neutralization with 2 M KOH, acetate buffer (pH 5) was added to the *Amanita* spp. samples to reach a final volume of 100 mL. Next, 0.1 mL of the solution was taken, and exo-1,3-β-glucanase (20 U/mL) and β-glucosidase (20 U/mL) were added. The resulting solution was incubated in a water bath at 40 °C for 1 h. To each analyzed sample, 3 mL of Megazyme glucose assay reagent (glucose oxidase/peroxidase; GOPOD) was added, and the mixture was once again incubated at 40 °C for 20 min. The samples were analyzed using a Helios Beta UV/VIS spectrophotometer (Thermo Fisher, UK) at a wavelength of $\lambda = 510$ nm and compared to a blank. An enzyme assay method was used to detect 1,3-1,6-β-glucans, which is an effective method for quantifying β-glucans in fungi, and the standard error of the method is <5% (Megazyme© International, Ireland Ltd, Wicklow, Ireland) [23].

3.8. Bioelement analysis

The dried fruiting bodies and mycelia of the studied species were pulverized using a Pulverisette 14-ball mill (Merazet, Poznań, Poland). The samples were analyzed for elements: Mg, K, Na, Ca, Fe, Zn, Mn, and Cu. From each dried fungal specimen, 0.5 g samples were weighed and transferred to Teflon vessels containing 2 mL of 30% H_2O_2 and 6 mL of 65% HNO_3. The samples were then subjected to wet mineralization in a closed system using a Magnum II mineralizer (ERTEC-Poland, Wroclaw, Poland). The resulting mineralized solution was heated on a hotplate for 60 min at 120 °C to remove excess reagents. Subsequently, the samples were transferred to 10 mL flasks and diluted four times with distilled water. Flame atomic absorption spectrometry (FAAS) was used to determine the elemental content. An iCE3500 atomic absorption spectrometer (Thermo Scientific, Gloucester, UK) was used for all measurements.

3.9. Statistical analysis of results

The statistical analysis of results was performed using ANOVA analysis of variance, with the determination of homogeneous groups using Tukey's HSD test at $p \leq 0.05$. The analyses were conducted using Statistica version 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA).

4. Results

4.1. Content of organic compounds

Among the indole compounds, the highest concentration was observed for 5-hydroxy-L-tryptophan. In the mycelium of *A. muscaria*, its amount was 167 mg/100 g dry weight (d.w.), and in the fruiting bodies, it was 137 mg/100 g d.w. In the case of *A. pantherina*, these values were less than half (32.5–63.5 mg/100 g d.w.). The highest content of 5-methyltryptamine was found in the mycelium of *A. pantherina* at 12.8 mg/100 g d.w., whereas in other analyzed samples, the content was 2.21–5.94 mg/100 g d.w. The content of L-tryptophan was similar in the fruiting bodies of both species, varying between 26.8 and 31.6 mg per 100 g d.w. The mycelium of *A. muscaria* contained the lowest amount of this substance at 6.23 mg/100 g d.w., and melatonin was detected only in this material, at a concentration of 10.5 mg/100 g d.w. (Table 1).

Table 1. **Content of bioactive compounds in the mushroom material of *A. muscaria* and *A. pantherina* [mg/100 g d.w. ± SD] and for β-glucans [g/100 g d.w. ± SD]**

***Amanita* spp.** / **Bioactive compound**	***A. muscaria* mycelial cultures**	***A. muscaria* fruiting bodies**	***A. pantherina* mycelial cultures**	***A. pantherina* fruiting bodies**
Muscimol	0.051 ± 0.003^{a}	0.692 ± 0.091^{b}	0.051 ± 0.003^{a}	0.093 ± 0.002^{a}
Ibotenic acid	*	*	*	*
Ergosterol	*	75.1 ± 2.5 ^{a}	8.97 ± 3.07^{b}	18.2 ± 0.1^{c}
Ergosterol peroxide	*	*	*	*
Lovastatin	51.7 ± 0.9^{a}	3.64 ± 0.30^{b}	10.6 ± 0.4^{c}	1.83 ± 0.02^{d}
Ergothioneine	9.96 ± 0.80^{a}	19.2 ± 2.3^{b}	14.4 ± 0.4^{c}	2.68 ± 0.07^{d}
5-Hydroxy-L-tryptophan	164 ± 5^{a}	137 ± 5^{b}	63.5 ± 2.4^{c}	32.5 ± 0.1^{d}
L-Tryptophan	6.23 ± 0.91^{a}	31.6 ± 1.4^{b}	26.8 ± 0.6^{c}	28.6 ± 0.2^{c}
Tryptamine	0.061 ± 0.001 ^{a}	*	Nd	Nd
Melatonin	10.5 ± 1.8^{a}	Nd	*	*
5-Methyltryptamine	5.94 ± 0.34^{a}	4.64 ± 0.11^{b}	12.8 ± 0.3^{c}	2.21 ± 0.01^{d}
β-Glucans	6.24 ± 0.09^{a}	5.78 ± 0.12^{b}	9.84 ± 0.12^{c}	6.13 ± 0.06^{d}

$n = 9$; *—trace amount; Nd—not detected; the letters a,b,c,d next to values represent Tukey's HSD post hoc results ($p < 0.05$)

The highest ergothioneine content was found in *A. muscaria* fruiting bodies at 19.2 mg/100 g d.w., while the lowest was detected in *A. pantherina* fruiting bodies at 2.68 mg/100 g d.w. Lovastatin content was highest in the mycelium of *A. muscaria* at 51.7 mg/100 g d.w., with other samples containing at least five times less. The highest

amount of ergosterol was found in the fruiting bodies of *A. muscaria* at 75.1 mg/100 g d.w., while the values in other samples were significantly lower. The contents of ergothioneine, lovastatin, and ergosterol showed statistically significant differences in all tested samples. The content of β-glucans was highest in the case of *A. pantherina* mycelium at 9.84 g/100 g d.w. while for other objects, the difference in values was not statistically significant. Some amount of muscimol, characteristic of *Amanita* spp., was detected in all tested samples. The highest amount of this substance was found in the fruiting bodies of *A. muscaria* at 0.692 mg/100 g d.w., whereas the other tested samples had smaller amounts of this substance. Trace amounts of ibotenic acid were also detected (at the limit of quantification) (Table 1).

4.2. Microelement and macroelement content

The results for bioelement content are shown in Table 2. The highest Fe content was observed in the fruiting bodies of *A. muscaria* (53.4 mg/100 g d.w.). The amount for other samples was more than twice as low and statistically significantly different in all cases. The Cu content was at a similar level in the fruiting bodies of both studied species (3.30–4.13 mg/100 g d.w.). Mycelial cultures contained significantly lower amounts of this element. The fruiting bodies of *A. pantherina* contained the highest Zn content at 68.9 mg/100 g d.w., whereas other tested materials contained less than half this amount. Mn content was highest in *A. muscaria* mycelium at 9.16 mg/100 g d.w., and lowest in *A. pantherina* fruiting bodies at 0.838 mg/100 g d.w.

Table 2. **Content of selected bioelements in *A. muscaria* and *A. pantherina* species [mg/100 g d.w. ± SD]**

Bioelement	*A. muscaria* mycelial cultures	*A. muscaria* fruiting bodies	*A. pantherina* mycelial cultures	*A. pantherina* fruiting bodies
Ca	4.70 ± 0.26^{a}	7.03 ± 0.10^{b}	8.16 ± 0.06^{b}	44.0 ± 3.3^{b}
Cu	0.412 ± 0.037^{a}	3.30 ± 0.24^{b}	0.575 ± 0.033^{c}	4.13 ± 0.13^{b}
Fe	36.4 ± 0.5^{a}	53.4 ± 0.2^{b}	24.1 ± 0.2^{c}	18.1 ± 1.1^{d}
K	497 ± 19^{a}	157 ± 1^{b}	637 ± 38^{a}	156 ± 1^{c}
Mg	$152 \pm 6^{a,c}$	$114 \pm 4^{b,c}$	$119 \pm 3^{a–c}$	107 ± 4^{d}
Mn	9.16 ± 0.04^{a}	4.55 ± 0.14^{b}	3.35 ± 0.17^{c}	0.838 ± 0.059^{d}
At	79.5 ± 2.0^{a}	18.5 ± 0.5^{b}	75.8 ± 1.4^{c}	6.65 ± 0.13^{d}
Zn	13.4 ± 0.3^{a}	6.19 ± 0.27^{b}	26.7 ± 0.5^{c}	68.9 ± 3.1^{d}

$n = 9$; the letters a,b,c,d next to values represent Tukey's HSD post hoc results ($p < 0.05$)

The highest K content was determined in the mycelium of *A. pantherina* at 637 mg/100 g d.w. It was significantly lower in the mycelium of *A. muscaria*, at 497 mg/100 g d.w., as well as in the fruiting bodies of both species. Although Mg content was at a similar level across all samples, the differences were statistically

significant. The highest Mg content was detected in the mycelium of *A. muscaria* (152 mg/100 g d.w.), while the lowest was found in the fruiting bodies of *A. pantherina* (107 mg/100 g d.w). On the other hand, Ca content was the highest in the fruiting bodies of *A. pantherina*, at 44.0 mg/100 g d.w. In the case of Na, its content was similar in both mycelial samples (75.8 and 79.5 mg/100 g d.w.), but was several times lower in the fruiting bodies (Table 2).

5. Discussion of results

The biomass obtained from mycelial cultures of *A. muscaria* contained muscimol (Figure 1A), which was determined to be 0.05% of the dry weight. Considering the ease of mycelium production, this could offer an efficient method for obtaining muscimol, especially since research on microdosing this substance is currently underway [24,25]. Other authors have mainly reported substances such as muscarine at 0.02% of dry weight, ibotenic acid at about 1% d.w., muscimol at approximately 0.09% d.w., as well as trace amounts of tropane alkaloids. In a fresh fruiting body weighing between 50 – 70 g, around 6 mg of muscimol and up to 70 mg of ibotenic acid may be present [26, 27]. The amounts of muscimol determined in the present study are consistent with previous analyses, while in contrast to earlier reports in scientific literature, ibotenic acid was detected only in trace amounts (Table 1) [26, 27]. Interestingly, significantly higher contents of both muscimol and ibotenic acid in mushroom caps have previously been confirmed for both analyzed species [27]. Due to the potential toxic effects of these mushrooms, previous studies have not examined the content of biologically active compounds with health-promoting properties. Therefore, the analyses carried out in this study on the presence of non-hallucinogenic indole compounds, ergosterol, ergothioneine and lovastatin are the first mycochemical investigations of this kind carried out to date, adding significant scientific value to this work.

Muscimol is a potent agonist of the GABA (γ-aminobutyric acid) neurotransmitter receptors, primarily affecting $GABA_A$ receptors in the central nervous system (CNS). GABA is the primary inhibitory neurotransmitter in the brain, and muscimol's action on $GABA_A$ receptors results in sedative, antianxiety, and sleep-inducing effects. It can also induce relaxation and, in larger doses, a hypnotic or directly sleep-inducing state. The anxiolytic effect is achieved by enhancing inhibitory neurotransmission via GABA receptors in the brain. The effects of muscimol may vary depending on the dose, individual sensitivity, and environmental factors. Consumption of mushrooms containing muscimol can be dangerous and should be avoided due to the risk of not only poisoning but also the potential for serious side effects [28].

In addition, the study identified a number of other bioactive substances, including indole compounds that demonstrate antidepressant effects. Among these compounds, 5-hydroxy-L-tryptophan, most notably, serves as a precursor for serotonin, one of the most important neurotransmitters in the brain responsible for regulating mood, sleep

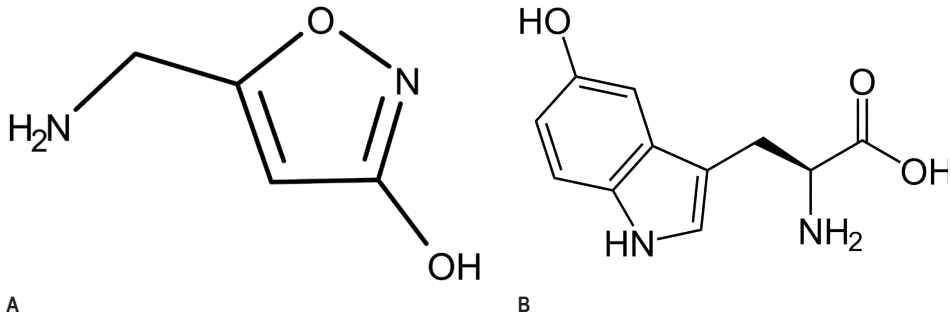

Figure 1. **Structural formulae: muscimol (A), 5-hydroxy-L-tryptophan (B)**

quality, and appetite [29]. Mushrooms are a rich source of indole compounds, among which melatonin is particularly noteworthy [30].

In the study, high contents of indole compounds were observed. In the mycelium of *A. muscaria*, the content of 5-hydroxy-L-tryptophan was 164 mg/100 g d.w., and a similarly high content was also found in the fruiting body of this species. In comparison to other species, this value falls within the average range. Depending on the species, the content of 5-hydroxy-L-tryptophan and other indole compounds varies from trace amounts to over 700 mg/100 g d.w., as evidenced in the case of *Pleurotus djamor* mycelium [31,32]. For *in vitro* cultures, conditions can be standardized and mycelial metabolism can be controlled by introducing various substances to the medium, including precursors for the synthesis of non-hallucinogenic indole derivatives, such as serine or anthranilic acid, or L-phenylalanine which is a precursor for the synthesis of phenolic acids [33,34]. Perhaps with the use of other ingredients and additives, such as elemental salts, it may be possible to obtain raw material enriched with the desired active ingredient. A similar relationship has been observed in the cultivation of fungi such as *Agaricus bisporus* and *Pleurotus* spp. [16,35].

Other compounds, such as ergothioneine, lovastatin, and ergosterol, were also identified in the extracts obtained from the mycelium and fruiting bodies of *A. muscaria* and *A. pantherina.* Ergothioneine is an amino acid that is a histidine derivative with strong antioxidant properties Some researchers suggest that it should be counted as a vitamin, as it is essential to the functioning of the human body and can only be acquired from food [36]. In the study of the selected species, high levels of ergothioneine were determined, with the highest concentration found in the fruiting bodies of *A. muscaria* (19.2 mg/100 g d.w.). Mushrooms are considered one of the best sources of this substance, as evidenced by its quantity in other edible species, such as *Pleurotus* spp. [31,37]. Lovastatin, a compound that regulates cholesterol metabolism in humans, helps lower the level of its harmful form LDL (Low-Density Lipoprotein) while maintaining the level of "good" cholesterol HDL (High-Density Lipoprotein). Mushrooms are considered one of the best sources of this substance [37]. In the study, the lovas-

tatin content in *A. muscaria* mycelium was 51.7 mg/100 g d.w., which is a relatively high amount. In comparison, the content of this compound was 67.89 mg/100 g d.w. in the fruiting bodies of *Cantharellus cibarius* and 28.84 mg/100 g d.w. in *Pleurotus citrinopileatus.* [31,37].

Sterols are compounds commonly found in mushrooms, which are recognized as a rich source of these compounds. The sterol group includes ergosterol, which is a precursor to vitamin D [38]. *Agaricus bisporus* (white button mushroom) is considered to be a particularly rich source of this compound, with a content of 61.5 mg of ergosterol per 100 g d.w. [39]. Studies have shown that the fruiting bodies of *A. muscaria* are also a good source of this substance. A group of compounds, studied extensively for biological activity, are polysaccharides, especially β-glucans, which are part of the fungal cell walls. These compounds, with proven immunomodulatory properties, are used in cancer therapies as adjuvants during standard chemotherapy and radiotherapy, particularly in China and Japan [40]. The study revealed the presence of β-glucans in all the samples tested, but comparing their content with that of other species is difficult and may not always be meaningful, due to the unique activity of these polysaccharides in specific fungal species [23]. In a study conducted in 1992, β-glucans contained in *A. muscaria* were observed to exhibit inhibitory activity against the proliferation of cancerous tumors *in vitro*. Additionally, the anticancer potential of extracts containing *A. muscaria* polysaccharides against melanoma was also demonstrated [41,42].

Mushroom fruiting bodies are a source of many macro – and micronutrients essential for proper human functioning. Mushrooms contain, among other components, essential elements such as K, Ca, Mg, Zn, Na, Se, and Fe in amounts that suggest they may be a valuable source of these nutrients, and their consumption may prove to be an important source of nutritional supplements [38,43]. The content of these elements is highly variable and depends on the growing environment and the substrate on which the mycelium develops. In the case of *in vitro* cultures, various bioelements can be easily modified and added, thereby altering fungal metabolism and allowing for the production of more easily absorbable organic forms of scarce elements, such as selenium [44]. The macro – and micronutrient contents obtained in this study are similar to those reported in previous scientific investigations using edible mushrooms. The research results demonstrated high levels of Fe, Mn, Zn, Mg, and K, although these values were subject to variation depending on the specific raw material studied (Table 2). With respect to previously determined bioelements for *A. muscaria*, particularly large differences were observed in the contents of such bioelements as Ca (136.7 mg/100 g d.w., and now 7.03 mg/100 g d.w.), Fe (11.8 mg/100 g d.w., and now 53.4 mg/100 g d.w.) and Zn (12.9 mg/100 g d.w., and now 6.19 mg/100 g d.w.). In contrast, for Mg and Cu, the amounts remained at similar levels (130 and 114 mg/100 g d.w., and 3.84 and 3.30 mg/100 g d.w., respectively). Similar concentrations of Fe and Cu were determined for *A. pantherina* (16.5 and 18.1 mg/100 g d.w. and 4.52 and 4.13 mg/100 g d.w., respectively), while Zn and Mg were higher in the current experiment (16.5 and 68.9 mg/100 g d.w. and 103.9 and 156 mg/100 g d.w., respectively). A difference was

also observed for Ca (84.2 mg/100 g d.w. in previous studies and 44.0 mg/100 g d.w. now) [45].

In summary, the mycelia of the studied species are a potential source of muscimol. In addition, they do not contain toxic ibotenic acid. A number of substances with health-promoting properties were identified in both the fruiting bodies and the mycelium of *A. muscaria* and *A. pantherina*. Notably, the mycelium of *A. muscaria* contained higher levels of active compounds in most cases when compared to *A. pantherina*.

6. Conclusion

Based on the results, it can be inferred that the mycelium of *A. muscaria* and *A. pantherina* constitutes a potential raw material rich in substances that influence the human nervous system. Muscimol and the identified indole compounds may be used in the future for the development of antidepressant formulations, but further *in vitro*, *in vivo*, and clinical studies are needed. For the first time, high levels of other compounds with proven health-promoting properties, such as lovastatin, ergosterol and ergothioneine, have been determined, further increasing the potential therapeutic value of the mycelium of *A. muscaria* and *A. pantherina.* However, large-scale, much more complex, and long-term investigations are necessary before a drug based on *Amanita* spp. can be considered for practical use. Importantly, such a drug should not be classified as a dietary supplement due to its content of the psychoactive compound muscimol.

This research highlights the complex nature of studying *A. muscaria* and *A. pantherina*, particularly in the context of mycelial cultures as a potential therapeutic agent. In conclusion, it should be emphasized that not only the fruiting bodies obtained from their natural environment but also mycelia derived from *in vitro* cultures could serve as standardized mushroom material for future studies. These materials could be used to investigate the therapeutic potential of muscimol and indole derivatives in treating depression as well as other CNS disorders.

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