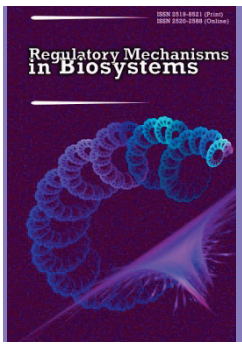

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Effect of the composition of a biologically active dietary supplement with macrofungi mycelia on its antioxidant activity

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The global demand for macrofungi is driven by their rich content of biologically active substances and trends such as the need for natural medicines, the growing popularity of vegan diets, increased consumer interest in therapeutic and prophylactic dietary supplements, and the expanding use of fungi in the food, pharmaceutical, and cosmetic industries. The aim of this study was to investigate the effect of the composition of a biologically active dietary supplement based on a mixture of macrofungal mycelium on the total phenolic content and antioxidant activity of the supplement. The mycelium of *Fomitopsis pinicola*, *Pleurotus ostreatus* and *Trametes versicolor* were obtained through controlled submerged static cultivation on suitable media. The powdered mycelia of *F. pinicola*, *P. ostreatus*, and *T. versicolor*, which are cubic and plate-shaped conglomerates with rounded edges, translucent, with a smooth surface, were mixed with excipients and granulated. Pharmacotechnological characteristics such as compressibility, powder flowability, moisture content, as well as dosage uniformity and disintegration time of hard capsules significantly varied depending on the granulate composition. The total phenolic content and antioxidant activity of each mycelium and their mixture, as well as of the selected granulates, were determined. Differences in the total phenolic content and antioxidant activity of each mycelium and their mixture were insignificant. Determination of these parameters and the pharmacotechnological parameters of the granulates allowed the identification of the most suitable excipients: lactose monohydrate (5%), mannitol:microcrystalline cellulose 101 in the ratio 2:1 (10%), sodium croscarmellose (1%), and a 0.7% solution of carboxymethyl cellulose (4%). This mixture contains 29.83 ± 0.49 mg GAE/g of total phenolic content and can neutralize the DPPH free radical scavenging by 86.53 ± 0.62%. The obtained results of the study confirm the prospects of using macrofungi mycelium of *F. pinicola*, *P. ostreatus*, and *T. versicolor* as a promising raw material for the development of a new dietary supplement with antioxidant activity.

Keywords: fungal biomass; total phenolic content; DPPH scavenging ability; nutraceutic; wet granulation; pharmacotechnological indicators.

Introduction

Macrofungi have been used in medicine and pharmacy for centuries due to their wide range of activities, including antimicrobial, immunomodulatory, antioxidant, antiviral, hypotensive, neuroprotective, and anti-inflammatory effects (Chugh et al., 2022; Ambhore et al., 2024; Sun et al., 2024). Bioactive metabolites, such as polysaccharides, phenolic compounds, terpenes, peptides, alkaloids, steroids, and vitamins are responsible for these activities (Lu et al., 2020; Zhou et al., 2023; Martinez-Burgos et al., 2024). Over the past few decades, urbanization, military conflicts, food shortages, and other factors have significantly altered people's lifestyles and food preferences. These changes in dietary habits have contributed to a rise in lifestyle-related diseases. At the same time, interest in developing natural medicines and therapeutic agents, including those derived from macrofungi, has surged in the 21st century (Niego et al., 2021). The global market for dietary mushroom supplements is experiencing substantial growth, driven by increasing awareness of the health benefits of mushrooms. By 2024, the market is projected to be worth approximately USD 10.6 billion, with expectations to reach around USD 24.74 billion by 2026. Moreover, the broader mushroom market, encompassing both edible and medicinal varieties, is also showing strong growth. Projections indicate that it will reach USD 94.57 billion by 2028, expanding at a compound annual growth rate (CAGR) of 9.2% (www.thebusinessresearchcompany.com/report/mushroom-global-market-report). Examples of such products include: "The Real Thing – Medical Mushrooms 60s" (Wellness Warehouse, UK), "Mico-Mix" and "Mico-Five" (Hifas da Terra, EU), and "Agarikon.1" and "Mykoprotect.1" (MycoSan, Croatia). Wellness Warehouse's "The Real Thing – Medical Mushrooms 60s" contains a composition of *Agaricus blazei*, *Auricularia auricular*, *Cordyceps militaris*, *Cordyceps sinensis* (current name: *Ophyocordyceps sinensis*), *Coriolus versicolor* (current name: *Trametes versicolor*), *Ganoderma lucidum*, *Grifola frondosa*, *Inonotus obliquus*, *Hericium erinaceus*, *Lentinula edodes*, *Phellinus linteus*, *Pleurotus eryngii*, *P. ostreatus* that supports the immune system, promotes cytoprotective activity, and helps maintain healthy cellular function (www.wellnesswarehouse.com/the-real-thing-medical-mushrooms-60s-60s-00011120438103). "Mico-Mix" – the combination from the fruiting bodies of *Ganoderma lucidum*, *Grifola frondosa*, and *L. edodes* is the production of Hifas da Terra (EU)

(https://hifasdaterra.com/en/product/mico-mix-extract-of-3-mushrooms/). Extracts from the fruiting bodies of the mushrooms *A. blazei*, *G. lucidum*, *G. frondosa*, *I. obliquus*, and *L. edodes*, were used in the formulation of "Mico-Five" (http://hifasdaterra.com/ en/product/mico-five-vit-c). Croatian scientists from the Ruđer Bošković Institute and the Faculty of Food and Biotechnology at the University of Zagreb tested over 150 combinations of mushroom extracts to develop "Agarikon.1" – a medicinal mushroom extract formulation designed for patients with cancer and other critical conditions. "Agarikon.1" contains extracts from *A. blazei*, *G. lucidum*, *G. frondosa*, *L. edodes*, *P. ostreatus*, and *Trametes versicolor*, with the addition of vitamin C (http://mykosan. com/agarikon-1-medicinal-mushrooms-cancer). MycoSan's product such as "Mykoprotect.1", is a medicinal mushroom supplement formulated for patients with serious viral infections, including various types of hepatitis, HPV, herpes, HIV/AIDS, coronavirus, and the flu. Extracts from *G. lucidum*, *T. versicolor*, and *L. edodes*, along with the addition of vitamin C, ensure the optimal effectiveness of "Mykoprotect.1" (http://mykosan.com/ mykoprotect-1-antiviral-medicinal-mushroom-extract).

As of October 25, 2024, there are no registered macrofungi-based pharmaceutical products on the Ukrainian market, according to data from the State Expert Center of the Ministry of Health of Ukraine. However, the domestic pharmaceutical market, including pharmacies and online resources, is gradually expanding its range of natural mushroom products in response to the growing global demand for medicinal fungi. Currently, several dietary supplements (DS) based on macrofungi are offered by Ukrainian manufacturers. Leading companies in this field include NPO "PhytoBioTechnologies" (series "Mushroom First Aid Kit", Kyiv), LLC "Elit-Pharm" (Dnipro), the Kyiv Center for Fungotherapy, Bioregulation, and Ayurveda (Kyiv), and companies like "Mushroom Kingdom" (Kyiv), "Micofit" (Kharkiv), "Amanita" (Odesa), and "Dana, Ya" (Kyiv).

The foundation of Ukrainian mushroom products is based on basidiomycete species such as *A. blazei*, *Cantharellus cibarius*, *Coprinus comatus*, *Flammulina velutipes*, *G. lucidum*, *G. frondosa*, *H. erinaceus*, *I. obliquus*, *L. edodes*, *Pholiota nameko*, and *P. ostreatus*, along with the ascomycete fungus *O. sinensis*. The most well-known dietary supplement is "Mikoton", based on *F. fomentarius*, which is industrially produced by LLC "Mikoton-Aglikon" (Kyiv region). An analysis of these products shows that domestic manufacturers produce both solid (capsules, tablets, powders, herbal teas, dry extracts, suppositories) and liquid (drops, liquid extracts) forms. The raw materials used for producing these DS include the fruiting bodies and/or mycelium of mushrooms, which are rich in valuable natural biologically active substances.

It should be noted that people are becoming more proactive about their health, focusing on prevention rather than treatment (Waldman & Terzic, 2019). Mushroom-based supplements align with this trend, offering a way to incorporate natural, nutrient-dense products into daily routines. These supplements are often marketed for their general health benefits, such as boosting energy, reducing fatigue, and promoting overall well-being, which appeals to consumers seeking non-pharmaceutical wellness options (Risoli et al., 2023; Suberu et al., 2024). It should be noted that some studies suggest the possibility that macrofungi antioxidants may reduce the risk of certain diseases (Kozarski et al., 2015; Dávila Giraldo et al., 2023) or improve health (Kalaras et al., 2017; Liuzzi et al., 2023). The aim of this study was to investigate the effect of the composition of a biologically active dietary supplement based on a mixture of macrofungal mycelium on the total phenolic content (TPC) and antioxidant activity (AOA) of the supplement, and to establish the pharmaco-technological parameters for its dosage forms.

Materials and methods

The macrofungi species *Fomitopsis pinicola* 1523 (Sw.) P. Karst., *Pleurotus ostreatus* 551 (Jacq.) P. Kumm., and *Trametes versicolor* 353 (L.) Lloyd were kindly provided as pure cultures from the IBK Mushroom Culture Collection at the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (Bisko et al., 2020). Mycelia of *P. ostreatus* and *T. versicolor* were grown on a liquid nutrient medium containing CO_2-cake amaranth in a thermostat at 25 °C, without agitation. Mycelium of *F. pinicola* was cultured on a synthetic medium (composite-on per liter: 25.0 g xylose, 3.0 g yeast extract, 2.0 g peptone, 1.0 g K-HPO-, 1.0 g KH-PO-, 0.25 g MgSO-•7H-O) in a thermostat at 30 °C, also without agitation. After 14 days of cultivation, the mycelium of the fungi under study was separated from the medium by filtration through Whatman filter paper No. 4. It was then washed with distilled water, dried to a constant weight at 60 °C, and ground using a laboratory mill LZM-1 (Olis, Ukraine, 2009) for 30 seconds (Fig. 1).

F. pinicola *P. ostreatus* *T. versicolor*

Fig. 1. Ground up mycelium of the studied fungal species

The obtained powders were separately sieved through a standard set of woven-wire mesh analytical test sieves with a diameter of 20 cm using the dry sieving method (European Directorate for the Quality of Medicines & Healthcare of the Council of Europe, 2022). The results of particle-size distribution were evaluated by the difference in the weight of the sieve before and after the test with the powder.

The fungal biomass mixture (comprising *T. versicolor*, *P. ostreatus*, and *F. pinicola* in a 2.0:0.5:0.5 ratio) was analyzed for bulk density and tapped density according to section 2.9.34 of the European Pharmacopoeia (European Directorate for the Quality of Medicines & Healthcare, 2022). A powder density tester (Pharma Test PT-TD200, Germany, 2017) was used. Additionally, powder flow properties were assessed by calculating the compressibility index and Hausner ratio according to section 2.9.36 of the European Pharmacopoeia (European Directorate for the Quality of Medicines & Healthcare, 2022).

To develop granules with fungal mycelium, the following excipients were used: GranuLac® 200 lactose monohydrate (LMN) (Meggle, Germany); microcrystalline cellulose (MCC) 101 (Mingtai Chemical Co. LTD, Taiwan); mannitol (MNT) (Sfera Sim LLC, Ukraine); croscarmellose sodium (CCS) (Mingtai Chemical Co. LTD, Taiwan); magnesium stearate (Electrogasokhim LLC, Ukraine); talcum (Sfera Sim LLC, Ukraine); polyvinylpyrrolidone (PVP) K30 grade (StarTech & JRS Speciality Products Co. LTD, China); carboxymethyl cellulose (CMC) (Ingredio Company, Ukraine). The compositions of the studied formulations for encapsulation are shown in Table 1.

Table 1
Composition of the studied granulate formulations

Composition, %	Granulate formulations								
	1	2	3	4	5	6	7	8	9
Mycelium mixture of *T. versicolor*, *P. ostreatus,* and *F. pinicola* (2:0.5:0.5)	80	80	80	80	80	80	80	80	80
LMN	16	8	8	15	5	5	14	3	3
MCC 101	–	8	–	–	10	–	–	–	11
MNT:MCC 101 (2:1)	–	–	8	–	–	10	–	11	–
CCS	1	1	1	1	1	1	1	1	1
7 % PVP K30	3	–	–	4	–	–	5	–	–
15 % PVP K30	–	3	–	–	4	–	–	5	–
0.7 % CMC	–	–	3	–	–	4	–	–	5

Note: LMN – lactose monohydrate, MCC 101 – microcrystalline cellulose, MNT – mannitol, CCS – croscarmellose sodium, PVP K30 – polyvinylpyrrolidone, CMC – carboxymethyl cellulose.

Granules were obtained by wet granulation method using laboratory granulator NG-12 (Adonis LLC, Ukraine, 2002) with mesh diameter of the sieves 2 and 1 mm. The resulting granules were dried at 50 °C for 3 hours in a convective dryer. The granules after calibration were mixed with 1% magnesium stearate and 1% talcum.

The moisture content of the granulate was determined by the weight method after drying to a constant weight.

Hard granule-filled gelatin capsules of size 0 (Zhejiang Pujiang Enerkang Capsule Co., Ltd, China) were prepared with a manual encapsulator designed for 100 cells (HPO "Gidromash-1", Ukraine, 2018).

Uniformity of mass was evaluated according European Directorate for the Quality of Medicines & Healthcare of the Council of Europe (2022). The disintegration of capsules was assessed using a powder density tester (Pharma Test PT-TD200, Germany, 2017) according to section 2.9.1 of the European Pharmacopoeia (European Directorate for the Quality of Medicines & Healthcare, 2022). Purified water at 37 ºC was used as the liquid medium.

Ground up mycelium as well obtained granulates was extracted with purified water (1:10 v/v) under orbital shaking at 100 rpm for 48 hours at room temperature. After centrifuging for 10 minutes at 4500 rpm, the supernatant was collected and filtered through a 25 µm pore size filter (class 4 filter paper). The sample was then stored at 4 °C and directly analyzed for antioxidant activity (AOA) and total phenolic content (TPC).

AOA and TPC assay. AOA was assessed using the widely employed stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Blois, 1958; Gulcin & Alwasel, 2023). TPC was quantified using the Folin-Ciocalteu assay and expressed as gallic acid equivalents (GAE) (Folin & Ciocalteau, 1927; Singleton & Rossi, 1965). The detailed procedures for both analyses were provided in our previous study (Krupodorova et al., 2024).

Each experimental measurement was carried out in five replicates, and the data are presented as mean ± standard deviation (x ± SD). A one-way analysis of variance (ANOVA) with Tukey's test, applying a Bonferroni adjustment for multiple comparisons, was used to assess the effect of granulate composition on the pharmacotechnological properties of a fungal mixture, TPC and antioxidant activity AOA of fungal mycelia as well as fungal mixture, with significance set at $P < 0.05$.

Results

Particle characterization and antioxidant potential of fungal mycelium for supplement development. The fraction with the highest quantitative content in the analyzed powders of fungal mycelia was separated through grinding and sieving. Optical microscopy allowed the assessment of the shape of the studied mycelium particles. All the powders are conglomerates of cubic and plate-like shapes with rounded edges, semi-transparent, and with smooth surfaces. When the mycelium was ground for 30 seconds, it produced powders with different levels of fineness (Fig. 2). For *T. versicolor* and *F. pinicola*, the characteristic particles primarily measured between 125–180 µm, which is classified as fine powder. In contrast, *P. ostreatus* exhibited particles ranging from 180–355 µm, classified as moderately fine powder. The small quantity of particles that fall outside the main fraction is minimal; however, this could adversely affect the technological properties of the biomass powder mixture, particularly its flowability.

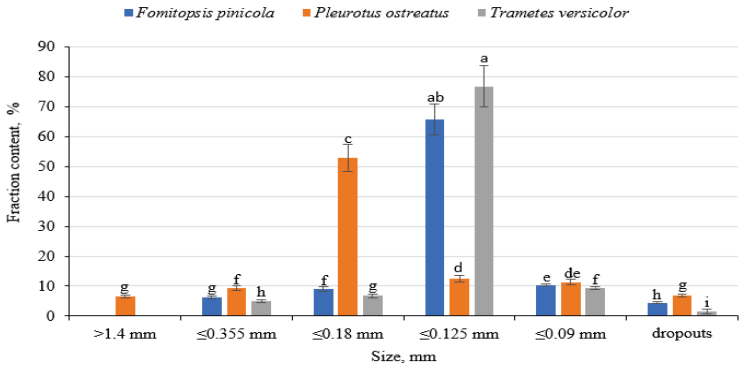

Fig. 2. Fractional composition of ground up mycelium of *F. pinicola*, *P. ostreatus* and *T. versicolor* (x ± SD, n = 5): different letters indicate samples that differ significantly from each other within the graphs ($P < 0.05$) according to Tukey's test with a Bonferroni correction

The fungal mycelium was analyzed for AOA and TPC (Table 2). All the samples studied demonstrated a high level of free radical inhibition and had a high TPC. The dry ground up mixture of mycelium from *T. versicolor*, *P. ostreatus*, and *F. pinicola*, in a ratio of 2.0:0.5:0.5, obtained under controlled and suitable cultivation conditions, has been chosen as the basis for the future biologically active dietary supplement.

An evaluation of the mixture of *T. versicolor*, *P. ostreatus*, and *F. pinicola* mycelium in powder form was conducted according to pharmacotechnological indicators (Table 3). The calculation of the compressibility index and the Hausner ratio was employed as one of the simplest, quickest, and most widely used techniques to evaluate powder flowability, serving as an indirect indicator of the material's physical and technological characteristics. For this method, it is essential that the initial bulk volume falls between 150 to 250 mL. However, the 100.0 g sample of the examined powder exhibited a notably lower volume than 150 mL. Consequently, a 100 mL cylinder with divisions of 1 mL was utilized for a sample weighing 50.0 g. The results obtained aligned with the values on the scale of flowability, which indicated poor flowability, as was expected. This outcome led us to continue the selection of excipients and technological approaches to enhance flowability.

Table 2
TPC and AOA in raw materials of fungi (x ± SD, n = 5)

Mycelium of fungi	TPC, mg GAE/g d.w.	DPPH inhibition, %
F. pinicola	35.00 ± 0.40^{c}	86.09 ± 0.33^{d}
P. ostreatus	38.97 ± 0.79^{b}	95.85 ± 0.76^{c}
T. versicolor	39.97 ± 0.99^{a}	98.31 ± 0.21^{b}
Mycelium mixture of *T. versicolor, P. ostreatus, F. pinicola* (2:0.5:0.5)	40.50 ± 0.51^{a}	99.61 ± 0.14^{a}

Note: different letters indicate samples that differ significantly from each other within the columm ($P < 0.05$) according to Tukey's test, with Bonferroni correction.

Formulation and bioavailability optimization of granules with fungal mycelium. Utilizing excipients, granulate samples containing fungal mycelium were created, and a pharmacotechnological assessment was conducted to attain the desired mass flowability necessary for encapsulation. This process aimed to identify the most suitable formulation and method for producing a solid dosage form, specifically hard capsules. The granules produced exhibited considerable variations in their physical properties. To comply with the methodological standards, samples of 75.0 and 100.0 g were measured for analysis. The calculated and observed values regarding bulk volume before and after powder mechanically tapping, along with the compressibility index and Hausner's ratio, primarily aligned with the Scale of Flowability, indicating passable, fair, and good flowability. Optimal outcomes were achieved by employing lactose monohydrate combined with a blend of mannitol (MNT) and microcrystalline cellulose (MCC) at a 2:1 ratio as fillers, alongside a 15% PVP solution serving as a binder solution (5%) – specifically in sample 8, and 0.7 % CMC solution serving as a binder solution (4%) – specifically in sample 6 (Table 3). The granulates exhibited considerable variations in their moisture levels. Since the highest moisture content values were recorded for the final samples (8 and 9), these samples will not be utilized in subsequent research.

Table 3
Pharmacotechnological properties of a mixture of *T. versicolor*, *P. ostreatus* and *F. pinicola* mycelium in the ratio of 2:0.5:0.5 and experimental samples of encapsulation masses with mycelium mixture (x ± SD, n = 5)

Composition of experimental samples	Compressibility index, %	Hausner ratio	Moisture, %
Mixture of *T. versicolor*, *P. ostreatus* and *F. pinicola* mycelium	28.27 ± 1.07	1.39 ± 0.04^{a}	1.24 ± 0.06^{h}
1. MM (80%)+LMN (16%)+CCS (1%)+7% PVP K30 (3%)	21.78 ± 0.91^{a}	1.28 ± 0.02^{b}	1.45 ± 0.07^{f}
2. MM (80%)+LMN (8%)+MCC 101 (8%)+CCS (1%)+15% PVP K30 (3%)	17.09 ± 0.35^{d}	1.20 ± 0.01^{d}	1.49 ± 0.03^{f}
3. MM (80%)+LMN (8%)+MNT:MCC 101 (8%)+CCS (1%)+0.7% CMC (3%)	18.01 ± 0.44^{c}	1.22 ± 0.03^{cd}	1.80 ± 0.11^{d}
4. MM (80%)+LMN (15%)+CCS (1%)+7% PVP K30 (4%)	22.45 ± 0.97^{a}	1.29 ± 0.01^{b}	1.11 ± 0.05^{g}
5. MM (80%)+LMN (5%)+ MCC 101 (10%)+CCS (1%)+15% PVP K30 (4%)	19.80 ± 0.62^{b}	1.25 ± 0.01^{c}	1.83 ± 0.08^{d}
6. MM (80)+LMN (5)+MNT:MCC 101 (10)+CCS (1)+0.7% CMC (4)	15.04 ± 0.09^{e}	1.18 ± 0.02^{d}	1.59 ± 0.12^{e}
7. MM (80%)+LMN (14%)+CCS (1%)+7% PVP K30 (5%)	21.62 ± 0.89^{a}	1.28 ± 0.01^{b}	1.98 ± 0.01^{c}
8. MM (80%)+LMN (3%)+MNT:MCC 101 (11%)+CCS (1%)+15% PVP K30 (5%)	11.20 ± 0.07^{f}	1.13 ± 0.02^{e}	2.62 ± 0.02^{a}
9. MM (80%)+LMN (3%)+MCC 101 (11%)+CCS (1%)+ 0.7% CMC (5%)	20.53 ± 0.73^{b}	1.26 ± 0.01^{bc}	2.29 ± 0.01^{b}

Note: MM – mycelium mixture of *T. versicolor*, *P. ostreatus*, *F. pinicola* (2:0.5:0.5), LMN – lactose monohydrate, MCC 101 – microcrystalline cellulose, MNT – mannitol, CCS – croscarmellose sodium, PVP K30 – polyvinylpyrrolidone, CMC – carboxymethyl cellulose; different letters indicate samples that differ significantly from each other within the columm ($P < 0.05$) according to Tukey's test, with Bonferroni correction.

The studied pharmacotechnological characteristics of the obtained hard capsules are presented in Table 4. Upon examining these findings, it is important to highlight the varying filling of hard capsules with the experimental encapsulation samples, which correlates directly with the bulk density of the granulate. In the context of hard capsule formulations with a mixture of *Trametes versicolor*, *Pleurotus ostreatus*, and *Fomitopsis pinicola* mycelium, disintegration time indicates how long it takes for the capsule containing this mycelium blend to disintegrate under conditions mimicking the gastrointestinal environment. Disintegration times ranged from 7 minutes 45 seconds to 22 minutes 35 seconds. The best disintegration time was recorded for sample No. 6 (7 minutes 45 seconds), which also exhibited the acceptable deviation in the uniformity of dosage for hard capsules. This metric is essential for ensuring that the active compounds in the fungal mycelium are effectively released and can be absorbed by the body. The disintegration time of a capsule influences its effectiveness, as it impacts the release and bioavailability of the active ingredients.

Table 4
Pharmacotechnological properties of hard capsules` experimental samples with a mixture of *T. versicolor*, *P. ostreatus* and *F. pinicola* mycelium

Composition of experimental samples	Average weight of hard capsule contents, g	Disintegration time, min
1. MM (80%)+LMN (16%)+CCS (1)+7% PVP K30 (3)	0.396 ± 0.011^{a}	22.35 ± 0.45^{a}
2. MM (80)+LMN (8)+MCC 101 (8)+CCS (1)+15% PVP K30 (3)	0.296 ± 0.006^{d}	12.51 ± 0.20^{c}
3. MM (80)+LMN (8)+MNT:MCC 101 (8)+CCS (1)+0.7% CMC (3)	0.303 ± 0.005^{c}	12.28 ± 0.44^{c}
4. MM (80)+LMN (15)+CCS (1)+7% PVP K30 (4)	0.380 ± 0.010^{a}	17.34 ± 0.24^{b}
5. MM (80)+LMN (5)+ MCC 101 (10)+CCS (1)+15% PVP K30 (4)	0.396 ± 0.006^{a}	10.05 ± 0.35^{d}
6. MM (80)+LMN (5)+MNT:MCC 101 (10)+CCS (1)+0.7% CMC (4)	0.302 ± 0.006^{c}	7.45 ± 0.15^{e}
7. MM (80)+LMN (14)+CCS (1)+7% PVP K30 (5)	0.372 ± 0.008^{b}	10.14 ± 0.90^{d}

Note: see Table 3.

Evaluation of antioxidant activity and phenolic content in mycelium-based granulate formulations. The selected granulate compositions were analyzed for TPC (Fig. 3a) and AOA (Fig. 3b). Granulate samples 1, 2, and 4–7 had high TPC levels, with no statistically significant differences observed among samples 2 and 4–7. The lowest TPC was found in sample 3. The antioxidant activity of the samples studied varied significantly. The highest free radical inhibition activity was found in sample 2 and the lowest was observed in sample 4. Given the good pharmacotechnical properties and biological activities of the experimental samples of capsule masses with mycelium mixture, we consider the composition of granules formulation 6 to be the optimal.

Discussion

The use of microbiological synthesis products as active pharmaceutical ingredients is one of the promising directions in biotechnology and pharmaceutical development. Today, products containing rectified extracts, mycelium or fruiting bodies of medicinal mushrooms are classified as a separate group of dietary supplements, and their production must comply with good manufacturing practice (GMP) requirements. Macrofungi, including their mycelial forms, are a largely untapped resource for novel drug development (Niego et al., 2021; Sharif et al., 2024). Unlike traditional pharmaceutical production methods that often rely on synthetic chemical processes or extensive plant cultivation, fungal mycelium can be grown efficiently in controlled nutrient media. Its advantages include a natural origin, lower likelihood of side effects (Chen et al., 2018; Jeitler et al., 2020; Chugh et al., 2022), and safer production process compared to synthetic compounds. Additionally, like medicinal plants, fungal mycelium contains a unique complex of biologically active substances that deliver effective therapeutic outcomes (Sharif et al., 2024; Sun et al., 2024). Antioxidant activity is a central therapeutic feature of mushrooms, as oxidative stress underlies many chronic diseases, including cancer, cardiovascular

disease, and neurode-generative conditions (Abo Nahas et al., 2021). Portuguese scientists studied the antioxidant activity of a number of mushrooms, evaluating it taking into account the individual activity of each mushroom and their mixtures. Three variants of interaction of mushrooms in the mixture were observed: synergistic, additive and negative synergistic effects, with the synergistic variant prevailing (Queiros et al., 2009). Numerous compounds isolated from macrofungi have antioxidant properties: phenolic compounds, vitamins, polysaccharides, peptides, proteins, organic acids, carotenoids, alkaloids and nucleotides (Stajic et al., 2013). Bach et al. (2019) noted in their review that synergistic interactions of compounds can enhance the biological activity of individual components, leading to natural extracts being more effective than isolated bioactive compounds.

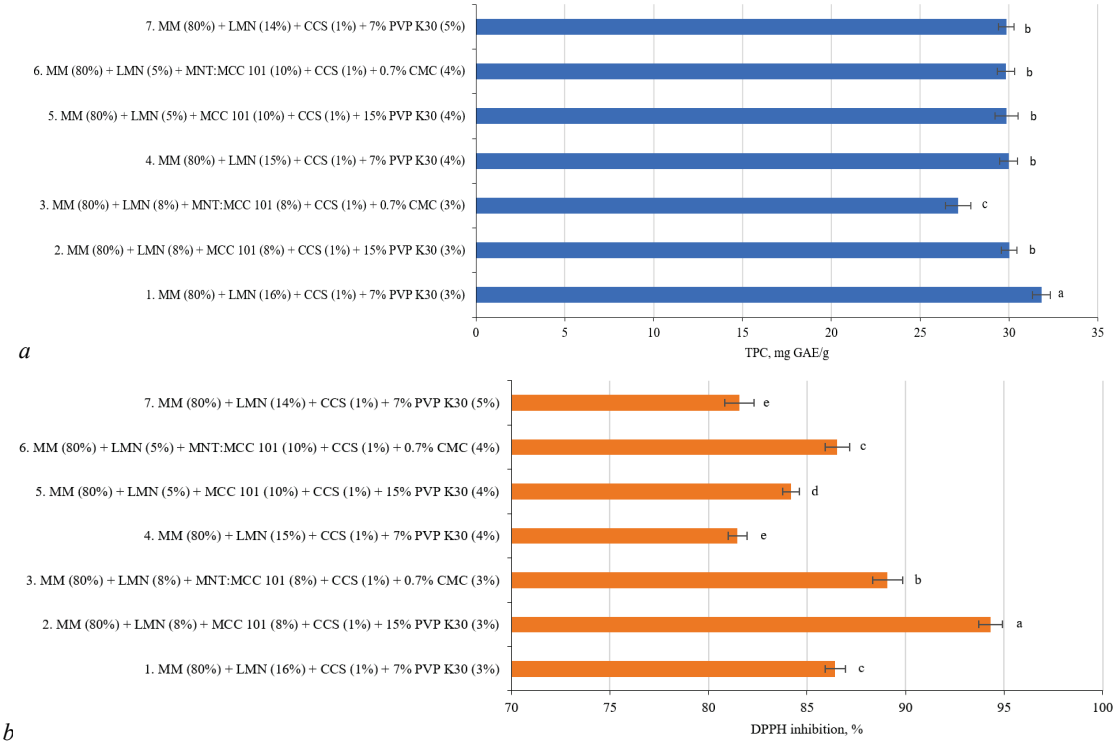

Fig. 3. Effect of granulate composition on TPC (*a*) and AOA (*b*) of dietary supplement (x ± SD, n = 5): composition of experimental samples: MM – mycelium mixture of *T. versicolor*, *P. ostreatus*, *F. pinicola* (2:0.5:0.5), LMN – lactose monohydrate, MCC 101 – microcrystalline cellulose, MNT – mannitol, CCS – croscarmellose sodium, PVP K30 – polyvinylpyrrolidone, CMC – carboxymethyl cellulose; different letters indicate samples that differ significantly from each other within the graphs ($P < 0.05$) according to Tukey's test with a Bonferroni correction

The study of Gebreyohannes et al. (2019) showed that the same extracts can exhibit different types of persistent activity, such as antioxidant and antimicrobial. Polyphenols in macrofungi play a significant role in their antioxidant potential (Stajic et al., 2013), making them valuable for health-promoting applications and possibly protecting against oxidative stress-related diseases. It has been found that polyphenols of macrofungi can neutralize free radicals, unstable molecules that can cause oxidative stress and damage to cells and tissues. By donating electrons to these free radicals, polyphenols stabilize them, reducing oxidative stress (Kozarski et al., 2015).

The mycelium of the used fungal species, as well as their mixture, had a high level of AOA and TPC. The fast similar phenolic content and antioxidant activity observed in the mycelium of individual mushroom species and their mixtures likely result from shared phenolic compounds, additive or compensatory effects within the mixtures. Also, if the individual fungi share a similar profile of these compounds, then their TPC and AOA can be similar, whether measured in isolation or in a mixture. On the other hand, further research is needed to understand the phenolic profile of fungi and their mixtures, as well as to identify specific polyphenolic compounds. The possibility of a "ceiling effect" should also be considered (Schünemann et al., 2002; Gouzi et al., 2019). Antioxidant assays often measure the ability to scavenge free radicals or reduce oxidants up to a certain limit. If both the individual species and the mixture reach this maximum capacity, their measured antioxidant activity might appear nearly identical, even if there are differences in less potent compounds or lower antioxidant concentrations. Our result is in line with the study by Huang et al. (2021), which reported nearly similar amounts of TPC and small differences in DPPH scavenging ability in hot water extracts for various combinations of mycelium from two to five different basidiomycete species. Our research is also consistent with other studies that have identified antiradical activity and phenolic content in the mycelium of the fungal species studied: *Trametes versicolor* (Han et al., 2015; Park et al., 2015; Kim et al., 2021), *Pleurotus ostreatus* (Morris-Quevedo et al., 2017; Özdal et.al., 2019; Krupodorova et al., 2024). Despite the availability of relevant data on the TBA and AOA content in the fruiting bodies of *F. pinicola* (Sulkowska-Ziaja et al., 2012; Nowacka et al., 2015; Zhang et al., 2023), only one study of ours (Krupodorova et al., 2024) has been published so far, focusing on the phenolic content and antioxidant activity of this fungus's mycelium.

It is well known that flowability plays a critical role in ensuring that powders used in drug formulations meet the necessary standards for manufacturability and product quality. However, the poor flowability of dry powder mixtures of *T. versicolor*, *P. ostreatus*, and *F. pinicola* mycelium can result from various factors related to their physical properties (moisture content, particle size and shape, density) and their fibrous nature. This prompted us to search for additional functional components to facilitate encapsulation.

In our work, we used well-known components with various functional purposes to develop granules. First of all, various fillers/diluents, such as lactose monohydrate (crystallized form of milk sugar), microcrystalline cellulose, the mixture of mannitol and microcrystalline cellulose.

Lactose monohydrate is widely used in the processes of creating granulated and tablet dosage forms by dry and wet granulation methods, due to good cohesive properties (promoting compressibility), narrow particle size distribution and mixing ability. Other types of diluents are microcrystalline cellulose and mannitol. Wang et al. (2023) studied the effect of diluent types, among which are those we used lactose monohydrate and microcrystalline cellulose (MCC) and granulation liquids on granule properti-

es for high shear wet granulation. The diluents' impact on granule properties was higher than that of granulation liquids. Different granulation methods (MCC with mannitol and MCC with dibasic calcium phosphate (DCP) were also studied by the Kittä et al. (2020). The tabletability of the MCC-DCP formulation decreased after wet granulation, while the tabletability of MCC-mannitol increased.

Junnila et al. (2022) varied the MCC-mannitol ratio to evaluate the optimal composition for high-dose formulations. The filler ratio MCC-mannitol had a significant effect on the tabletability of the model substances paracetamol and metformin, which have poor compaction properties. The flowability of the granules was not affected by the MCC-mannitol ratio. Another component of solid dosage forms – disintegrant (croscarmellose sodium (CCS) in our study) is added to the formulation to promote disintegration of granules and tablets to release the drug and its subsequent absorption. The effect of wet granulation on the efficacy of the disintegrant has been studied by Veronica et al. (2024). The disintegrants crospovidone (XPVP), croscarmellose sodium and sodium starch glycolate (SSG) were wet granulated together with dibasic calcium phosphate dihydrate as a poorly water-soluble matrix and polyvinylpyrrolidone as a binder. Köster & Kleinebudde (2023) investigated the influence of the localisation (intragranular, split or extragranular) of the same disintegrants (CCS, XPVP and SSG) on granules and tablets after twin-screw granulation. The authors found that disintegrants reduced particle size in granulation, with SSG having the least effect. Intragranular croscarmellose sodium and extragranular crospovidone were found to be advantageous for selected conditions, providing the fastest disintegration.

Polyvinylpyrrolidone (PVP) is a universal pharmaceutical excipient due to its inertness, non-toxicity and biocompatibility (Kurakula & Rao, 2020). It is also used in both for traditional and new formulations, acting as a coating and suspending agent, pore-forming agent, solubilized, stabilizer, etc. The main pharmaceutical applications of PVP are summarized in the review (Luo et al., 2021) improving the bioavailability and stability of drugs, improving the physical and mechanical properties of drugs, regulating the rate of drug release, and prolonging the circulation time of liposomes *in vivo*.

It should be noted that all the functional fillers in our test had a positive effect on the flowability of the experimental samples of encapsulants with mycelium mix. The best results were obtained with samples 6 and 8. Common to these samples was the addition of low levels of lactose monohydrate (3% and 5%) and increased levels of mannitol:MCC (10% and 11%). However, the addition of functional ingredients also affected the moisture content of the resulting granulates. A moisture content above 2% is typically undesirable for granules. This is due to a number of important factors: high moisture levels can cause granules to clump together, leading to poor flowability; excess moisture can affect the stability and quality of the granulate product; high moisture content creates a favourable environment for the growth of microorganisms, especially in our case where the granulate contains organic bases. Because of these potential risks, not all samples were tested in further experiments, but 1–7.

All tested granules contained phenolic compounds and were able to inhibit free radicals. The polyphenol content did not vary significantly, except for sample 3. The latter could hypothetically be the result of possible adsorption of fungal TPC, especially those that are more polar, which may adhere to MCC particles, which would reduce their detectability in analysis methods that require them to be in solution. The varying antiradical activity of the granulates tested may be attributed to the possible antioxidant activity of the added functional components. Mannitol showed scavenging effect toward hydroxyl free radicals (Wisselink et al., 2002; André & Villain, 2017; Häusler et al., 2021). Carboxymethyl cellulose also has a low antioxidant capacity (Moseley et al., 2003; Fan et al., 2014).

The research focused on development of a formula for a biologically active dietary supplement with antioxidant properties, based on a macrofungi mycelium complex does not address the identification of specific polyphenolic compounds and all potential risks associated with implementing this technology in a production setting. Additional studies using the manufacturer's available industrial equipment are required.

Future research efforts would involve comprehensive pharmacological studies to identify polyphenolic compounds in the proposed fungi mixture and to confirm the drug's efficacy and safety. Moreover, developing and standardizing the technological and regulatory documentation for its production will be essential. Also, future study might develop other dosage forms based on mycelium of *T. versicolor*, *P. ostreatus* and *F. pinicola*.

Conclusion

Utilizing fungal mycelium for the development of dietary supplements and subsequently pharmaceuticals represents a relevant, promising, and innovative approach. The results obtained indicate the potential for further phytochemical and pharmacological investigations of raw materials derived from fungal mycelium, aimed at developing natural products with antioxidant properties. Additionally, our study highlights a clear necessity to assess pharmacological and pharmacotechnological parameters for the formulation of dietary supplements that utilize mushroom mycelium. Determination of TPC, AOA and the pharmacotechnological parameters of the granulates identified an optimal composition of a biologically active dietary supplement, which comprised 80% of mycelia mixture, 5% lactose monohydrate, a 10% mixture of mannitol and microcrystalline cellulose in a 2:1 ratio, 1% sodium croscarmellose, and a 0.7% solution of carboxymethyl cellulose at 4%. This specific combination yielded a product with a total phenolic content of 29.83 ± 0.49 mg GAE/g and demonstrated a robust antioxidant capacity, effectively neutralizing 86.53 ± 0.62% of DPPH free radicals. These findings underscore the potential of this optimized excipient mixture for the formulation of effective mushroom-based dietary supplements that exhibit high TPC and antioxidant characteristics. With continuous advancements in research and technology, medications derived from mycelium could significantly influence the future of pharmacology.

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References

Abo Nahas, H. H., Darwish, A. M. G., Abo Nahas, Y. H., Elsayed, M. A., Abdel-Azeem, A. M., & Abdel-Azeem, M. A. (2021). Fungi as a gold mine of antioxidants. In: Abdel-Azeem, A. M., Yadav, A. N., Yadav, N., & Usmani, Z. (Eds.). Industrially important fungi for sustainable development. Springer Nature Switzerland AG, Cham. Pp. 73–113.

Ambhore, J. P., Adhao, V. S., Rafique, S. S., Telgote, A., Dhoran, R. S., & Shende, B. A. (2024). A concise review: Edible mushroom and their medicinal significance. Exploration of Foods and Foodomics, 2, 183–194.

André, P., & Villain, F. (2017). Free radical scavenging properties of mannitol and its role as a constituent of hyaluronic acid fillers: A literature review. International Journal of Cosmetic Science, 39(4), 355–360.

Bach, F., Zielinski, A. A. F., Helm, C. V., Maciel, G. M. M., Pedro, A. C., Stafussa, A. P., Ávila, S., & Haminiuk, C. W. I. (2019). Bio compounds of edible mushrooms: *In vitro* antioxidant and antimicrobial activities. LWT, 107, 214–220.

Bisko, N. A., Lomberg, M. L., Mytropolska, N. Y., & Mykhaylova, O., B. (2016). The IBK Mushroom Culture Collection. Alterpres, Kyiv.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature, 181(4617), 1199–1200.

Chen, S. N., Chang, C. S., Chen, S., & Soni, M. (2018). Subchronic toxicity and genotoxicity studies of *Antrodia* mushroom β-glucan preparation. Regulatory Toxicology and Pharmacology 92, 429–438.

Chugh, R. M., Mittal, P., Mp, N., Arora, T., Bhattacharya, T., Chopra, H., Cavalu, S. & Gautam, R. K. (2022). Fungal mushrooms: A natural compound with therapeutic applications. Frontiers in Pharmacology, 13, 925387.

Dávila Giraldo, L. R., Pérez Jaramillo, C. C., Méndez Arteaga, J. J., & Murillo-Arango, W. (2023). Nutritional value and antioxidant, antimicrobial and cytotoxic activity of wild macrofungi. Microorganisms, 11(5), 1158.
Fan, L., Peng, M., Zhou, X., Wu, H., Hu, J., Xie, W., & Liu, S. (2014). Modification of carboxymethyl cellulose grafted with collagen peptide and its antioxidant activity. Carbohydrate Polymers, 112, 32–38.
Folin, O., & Ciocalteau, V. (1927). On tyrosine and tryptophane determinations in proteins. The Journal of Biological Chemistry, 73(2), 627–650.
Gebreyohannes, G., Nyerere, A., Bii, C., & Sbhatu, D. B. (2019). Investigation of antioxidant and antimicrobial activities of different extracts of *Auricularia* and *Termitomyces* species of mushrooms. The Scientific World Journal, 2019, 7357048.
Gouzi, F., Maury, J., Heraud, N., Molinari, N., Bertet, H., Ayoub, B., Blaquière, M., Bughin, F., De Rigal, P., Poulain, M., Pincemail, J., Cristol, J. P., Laoudj-Chenivesse, D., Mercier, J., Préfaut, C., Pomiès, P., & Hayot, M. (2019). Additional effects of nutritional antioxidant supplementation on peripheral muscle during pulmonary rehabilitation in COPD patients: A randomized controlled trial. Oxidative Medicine and Cellular Longevity, 2019, 5496346.
Gulcin, I., & Alwasel, S. H. (2023). DPPH radical scavenging assay. Processes, 11(8), 2248.
Han, S. R., Noh, M. Y., Lee, J. H., & Oh, T. J. (2015). Evaluation of antioxidant and antimicrobial activities of solvent extracts from *Coriolus versicolor*. Journal of the Korean Society of Food Science and Nutrition, 44(12), 1793–1798.
Huang, C. W., Hung, Y. C., Chen, L. Y., Asatiani, M., Elisashvili, V. I., Klarsfeld, G., Melamed, D., Fares, B., Wasser, S. P., & Mau, J. L. (2021). Chemical composition and antioxidant properties of different combinations of submerged cultured mycelia of medicinal mushrooms. International Journal of Medicinal Mushrooms, 23(8), 1–24.
Jeitler, M., Michalsen, A., Frings, D., Hübner, M., Fischer, M., Koppold-Liebscher, D. A., Murthy, V., & Kessler, C. S. (2020). Significance of medicinal mushrooms in integrative oncology: A narrative review. Frontiers in Pharmacology, 2020, 580656.
Junnila, A., Wikström, H., Megarry, A., Gholami, A., Papathanasiou, F., Blomberg, A., Ketolainen, J., & Tajarobi, P. (2022). Faster to first-time-in-human: Prediction of the liquid solid ratio for continuous wet granulation. European Journal of Pharmaceutical Sciences, 172, 106151.
Kalaras, M. D., Richie, J. P., Calcagnotto, A., & Beelman, R. B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. Food Chemistry, 15(233), 429–433.
Kim, M., Ahn, C., & Kim, C. (2021). Antioxidant activity of indigenous *Trametes* species in Korea. Korean Journal of Mycology, 49(4), 433–440.
Köster, C., & Kleinebudde, P. (2023). Evaluation of binders in twin-screw wet granulation – optimal combination of binder and disintegrant. European Journal of Pharmaceutics and Biopharmaceutics, 186, 55–64.
Kozarski, M., Klaus, A., Jakovljevic, D., Todorovic, N., Vunduk, J., Petrović, P., Niksic, M., Vrvic, M. M., & van Griensven, L. (2015). Antioxidants of edible mushrooms. Molecules, 20(10), 19489.
Krupodorova, T., Barshteyn, V., Tsygankova, V., Sevindik, M., & Blume, Y. (2024). Strain-specific features of *Pleurotus ostreatus* growth *in vitro* and some of its biological activities. BMC Biotechnology, 24, 9.
Krupodorova, T., Barshteyn, V., Dzhagan, V., Pluzhnyk, A., Zaichenko, T., & Blume, Y. (2024). Enhancement of antioxidant activity and total phenolic content of *Fomitopsis pinicola* mycelium extract. Fungal Biology and Biotechnology, 11, 18.
Kurakula, M., & Koteswara Rao, G. S. N. (2020). Pharmaceutical assessment of polyvinylpyrrolidone (PVP): As excipient from conventional to controlled delivery systems with a spotlight on COVID-19 inhibition. Journal of Drug Delivery Science and Technology, 60, 102046.
Kyttä, K. M., Lakio, S., Wikström, H., Sulemanji, A., Fransson, M., Ketolainen, J., & Tajarobi, P. (2020). Comparison between twin-screw and high-shear granulation – the effect of filler and active pharmaceutical ingredient on the granule and tablet properties. Powder Technology, 376, 187–198.
Liuzzi, G. M., Petraglia, T., Latronico, T., Crescenzi, A., & Rossano, R. (2023). Antioxidant compounds from edible mushrooms as potential candidates for treating age-related neurodegenerative diseases. Nutrients, 15(8), 1913.
Lu, H., Lou, H., Hu, J., Liu, Z., & Chen, Q. (2020). Macrofungi: A review of cultivation strategies, bioactivity, and application of mushrooms. Comprehensive Reviews in Food Science and Food Safety, 19(5), 2333–2356.
Luo, Y., Hong, Y., Shen, L., Wu, F., & Lin, X. (2021). Multifunctional role of polyvinylpyrrolidone in pharmaceutical formulations. AAPS PharmSciTech, 22(1), 34.
Martinez-Burgos, W. J., Montes Montes, E., Pozzan, R., Serra, J. L., Torres, D. O., Manzoki, M. C., Vieira, R. L., dos Reis, G. A., Rodrigues, C., Karp, S. G., & Soccol, K. R. (2024). Bioactive compounds produced by macromycetes for application in the pharmaceutical sector: Patents and products. Fermentation, 10(6), 275.
Morris, H. J., Beltrán, Y., Llaurado, G., Batista, P. L., Perraud-Gaime, I., García, N., Moukha, S., Bermúdez, R. C., Cos, P., Hernandez, E., & Diez, J. C. (2017). Mycelia from *Pleurotus* sp. (oyster mushroom): A new wave of antimicrobials, anticancer and antioxidant bio-ingredients. International Journal of Phytocosmetics and Natural Ingredients, 2017(2), 14.
Moseley, R., Walker, M., Waddington, R. J., & Chen, W. Y. J. (2003). Comparison of the antioxidant properties of wound dressing materials-carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. Biomaterials, 24(9), 1549–1557.
Niego, A. G., Rapior, S., Thongklang, N., Raspé, O., Jaidee, W., Lumyong, S., & Hyde, K. D. (2021). Macrofungi as a nutraceutical source: promising bioactive compounds and market value. Journal of Fungi, 7(5), 397.
Nowacka, N., Nowak, R., Drozd, M., Olech, M., Los, R., & Malm, A. (2015). Antibacterial, antiradical potential and phenolic compounds of thirty-one polish mushrooms. PLoS One, 10(10), e0140355.
Özdal, M., Gülmez, Ö., Gür Özdal, Ö., & Algur Ö. F. (2019). Antibacterial and antioxidant activity of mycelial extracts of different *Pleurotus* species. Food and Health, 5(1), 12–18.
Park, K. M., Kwon, K. M., & Lee, S. H. (2015). Evaluation of the antioxidant activities and tyrosinase inhibitory property from mycelium culture extracts. Evidence-based Complementary and Alternative Medicine, 616298.
Risoli, S., Nali, C., Sarrocco, S., Cicero, A. F. G., Colletti, A., Bosco, F., Venturella, G., Gadaleta, A., Gargano, M. L., & Marcotuli, I. (2023). Mushroom-based supplements in Italy: Let's open Pandora's box. Nutrients, 15(3), 776.
Queiros, B., Barreira, J. C. M., Sarmento, A. C., & Ferreira, I. C. F. R. (2009). In search of synergistic effects in antioxidant capacity of combined edible mushrooms. International Journal of Food Sciences and Nutrition, 60(6), 160–172.
Schünemann, H. J., McCann, S., Grant, B. J. B., Trevisan, M., Muti, P., & Freudenheim, J. L. (2002). Lung function in relation to intake of carotenoids and other antioxidant vitamins in a population-based study. American Journal of Epidemiology, 155(5), 463–471.
Sharif, M., Nawaz, A., Naeem, S., Saeeda, U. H., Farid, A., Zada, N. S., Shah, A. A., Badshah, M., Alharbi, M. T., Tarabulsi, M. K., Selim, S., & Khan, S. (2024). Pharmaceutical and nutraceutical importance of bioactive metabolites from macrofungi. Journal of Biological Regulators and Homeostatic Agents, 38(5), 3621–3643.
Singleton, V. L., & Rossi, J. L. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144–158.
Stajic, M., Vukojevic, J., Knežević, A., Duletic, S., & Milovanovic, I. (2013). Antioxidant protective effects of mushroom metabolites. Current Topics in Medicinal Chemistry, 13(21), 2660–2676.
Sulkowska-Ziaja, K., Muszynska, B., Motyl, P., Pasko, P., & Ekiert, H. (2012). Phenolic compounds and antioxidant activity in some species of polyporoid mushrooms from Poland. International Journal of Medicinal Mushrooms, 14(4), 385–393.
Suberu, S. A., Isikhuemhen, O. S., Ogundare, T. E., Ekunseitan, D. A., & Fasina, Y. O. (2024). Benefits of mushroom-based supplements on growth performance, immunocompetence, and meat quality in Poultry. Animals, 14(11), 1517.
Sun, X., Shi, Y., Shi, D., Tu, Y., & Liu, L. (2024). Biological activities of secondary metabolites from the edible-medicinal macrofungi. Journal of Fungi, 10(2), 144.
Veronica, N., Lee, E. S. M., Heng, P. W. S., & Liew, C. V. (2024). Functionality of wet-granulated disintegrant in comparison to directly incorporated disintegrant in a poorly water-soluble tablet matrix. International Journal of Pharmaceutics, 661, 124467.
Waldman, S. A., & Terzic, A. (2019). Health care evolves from reactive to proactive. Clinical Pharmacology and Therapeutics, 105(1), 10–13.
Wang, L. F., Zhao, L., Hong, Y. L., Shen, L., & Lin, X. (2023). Attribute transmission and effects of diluents and granulation liquids on granule properties and tablet quality for high shear wet granulation and tableting process. International Journal of Pharmaceutics, 642, 123177.
Wisselink, H. W., Weusthuis, R. A., Eggink, G., Hugenholtz, J., & Grobben, G. J. (2002). Mannitol production by lactic acid bacteria: A review. International Dairy Journal, 12(2–3), 151–161.
Zhang, Z. F., Wu, C., Wang, M., Chen, J. F., & Lv, G. Y. (2023). Chemical fingerprinting and the biological properties of extracts from *Fomitopsis pinicola*. Arabian Journal of Chemistry, 16(5), 104669.
Zhou, Y., Chu, M., Ahmadi, F., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. (2023). Comprehensive review on phytochemical profiling in mushrooms: Occurrence, biological activities, applications and future prospective. Food Reviews International, 40(3), 924–951.