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Research Advances in Secondary Metabolites and Biological Activities of Mushroom Genus Fungi

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Abstract: *Agaricus fungi are widely distributed in nature, their secondary metabolites with novel structures and significant biological activities, mainly including steroids, terpenoids, nitrogen compounds, sphingoids, aliphatic compounds and other structural types, some of them possessed anti-tumor, antibacterial, anti-neurolysin, anti-angiotensin-converting enzyme activities. The secondary metabolites and their biological activities of Agaricus fungi in recent 20 years were reviewed to provide theoretical basis for further research and new drug development.*

Keywords: *Agaricus fungi; secondary metabolites; biological activity*

1. Introduction

The genus Mushroom, also known as *Agaricus*, belongs to the order Umbelliferae, family Umbelliferae, and there are more than 300 species worldwide. Some of the fungi in the genus are edible, but some species are poisonous. For example, A. placomyces looks similar to some edible mushrooms, but it has an unpleasant odour and causes some gastrointestinal problems when consumed [1]. The active secondary metabolites of *A. placomyces* have been reported in the previous literature to be structurally novel and diverse, and this paper reviews the secondary metabolites and their bioactivities isolated from A. placomyces fungi in the last 20 years.

2. Steroids and terpenoids

Fungi of the genus Agaricus are rich in steroids and terpenoids, most of which are ergosterol derivatives.

In 2007, Petrova *et al*. isolated ergosterol peroxide for the first time from *A. placomyces* and *A. pseudopratensis* substrates and detected the presence of ergosterol in *A. placomyces* using GC-MS [1].In 2011, Munkhgerel *et al*. first isolated ergosterol and ergosterol peroxide from A. silvaticus Schaeff ex. Secr [2], and ergosterol peroxide was reported in the literature to have strong antitumour activity and anticomplement activity [3].In 2011, Ueguchi *et al*. isolated ergosterol and ergosterol peroxide from the *A. blazei* subspecies to isolate three new compounds, among which benzoylergostane showed strong cytotoxicity (IC_{50}=6.0±0.33 μmol/L) against HepG2 cells[4] . In 2016, Shimiz *et al*. isolated an ergosterol derivative Agarol from *A. blazei* subspecies, which had strong cytotoxicity (IC_{50}=6.0±0.33 μmol/L) against human cancer cells A549, MKN45, HSC-3 and HSC-4 with significant cytotoxicity and IC_{50} of 0.26 μg/mL, 0.34 μg/mL, 1.72 μg/mL and 1.94 μg/mL, respectively, and revealed the molecular mechanism by which the compound Agarol induces apoptosis in cancer cells[5] . In 2016, Ragasa *et al*. isolated an ergosterol derivative from *A. blazei* subspecies to isolate brassicasterol [6], which was reported to have anti-aging properties according to another study [7].In 2021, Misgiati *et al*. isolated ergosterol from *A. blazei* Murill n-hexane extract, which could induce apoptosis in *A. blazei* subspecies by inhibiting the G2/M phase through the inhibition of cell cycle enhancing the induction of apoptosis in MCF-7 cancer cells, and the antitumour activity assay showed a strong cytotoxicity with an IC_{50} value of 43.10 μg/ mL [8].

Between 1999 and 2002, Hirotani *et al.* isolated eight ergosteroids and seven blazeispirol derivatives from *A. blazei* Murill mycelium with loss of the A-ring, which is a novel skeleton based on the ergosteroid skeleton, in which C-14, C-22, and C-25 were oxidised to form the ketoacetal structure, which is named as "protoblazeispirane" skeleton [9]. In 2023, Hirotani *et al*. isolated

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agariblazeispirol A and B with a unique steroidal skeleton from the mycelium of *A. blazei*, which was derived from the blazeispirol analogues. A new skeleton was formed by the breakage of dioxygen in the skeleton and the migration of C-18 methyl group from position 13 to 14, and both compounds were able to overcome the drug resistance of mouse leukaemia cells P388/VCR to a certain extent [10].In 2005, Hirotani *et al*. isolated agariblazeispirol C from *A. blazei* mycelium again, and through the blazeispirol A and BF_3-OEt_2 synthesised the compound [11].

In 2008, Itoh *et al.* obtained a biosteroid blazein with antiproliferative properties from *A. blazei* Murrill subspecies and found that human lung cancer LU99 and gastric cancer KATO III cells apoptosed at a concentration of 200 μmol/L, which in turn inhibited cell growth [12]. In 2020, Wu *et al*. identified five steroidal compounds from *A. gennadii* substrates, namely 5α, 6α-epoxy-(22E, 24R)-ergosta-8(14),22-diene-3β,7α-diol,stigmast-5-en-3-ol,5α,8α-epidioxyergosta-6,22- dien-3β-ol, ergosterol, and cerevisterol, of which stigmast-5-en-3-ol was isolated from this genus for the first time [13].

In 2013, Zhao *et al*. identified three haemaranthane-type sesquiterpenes from *A. arvensis* mycelium, of which 11,12-dihydroxy-15-drimeneoic acid and 3α,11,15-trihydroxydrimene were new compounds and 3β,11,12- trihydroxydrimene are known compounds [14].

3. Nitrogenous compounds

Nitrogen-containing compounds are another representative class of compounds in the secondary metabolites of mushroom fungi, including nitrogen heterocycles, amino acids, sphingolipids and so on, which mainly have anti-inflammatory, antibacterial, anti-tumour and other biological activities.

3.1. Azocyclic

In 2004, Kimura *et al*. discovered sodium pyroglutamate from *A. blazei* substrates, which has strong anti-tumour and anti-metastatic effects, is an anti-angiogenic substance, and also possesses immunomodulatory activity in loaded mice [15].In 2008, Kohno *et al*. isolated extracts from *A. bisporus* substrates 2-amino-3*H*-phenoxazin-3-one, abbreviated as APO, which possesses significant anti-inflammatory and immunomodulatory properties and may provide promising therapeutic strategies for T-cell-mediated inflammatory autoimmune and bacterial-induced diseases [16]. In 2019, Tian *et al*. obtained five natural aryl hydrocarbon receptor modulators from *A. blazei* substrates, 2-aminobenzothiazole, 2-mercaptobenzothiazole, 2-hydroxybenzothiazole, 2,2′-dithiobis(benzothiazole), and 6-methylisoquinoline, four of which are benzothiazole derivatives, suggesting that *A. blazei* may be one of the dietary sources of natural aryl hydrocarbon receptor modulators [17].In 2020, Wu *et al*. obtained adenosine, 1-phenazinecarboxylic acid from *A. gennadii* substrates, with 1- phenazinecarboxylic acid was isolated for the first time from this genus [13].

3.2. Amino acids

In 2006, Janåk *et al*. identified and quantified agaritine, a γ-glutamyl-substituted arylhydrazine derivative from *A. bisporus* spores using LC-MS-MS, with an average content of 0.304 ± 0.003% [18].

3.3. Sphingolipids

In 1999, Jennemann *et al*. isolated four novel glyco-inositol-phosphate-sphingolipids from *A. bisporus* subspecies and *A. campestris* subspecies, all four glycolipids contained a phytosphingosine consisting of α-hydroxystilbeneic acid and α-hydroxy lignocellulosic acid [19].

3.4. Other nitrogenous compounds

In 1999, Berg *et al*. isolated a new diol, agaridiol, from *Agaricus sp*. mycelium, which showed moderate antimicrobial activity against Bacillus subtilis ATCC6633, with 200 mg of the compound per 9 mm agar pore producing a zone of inhibition with a diameter of 16 mm [20].In 2011, Munkhgerel *et al*. urea was isolated from *A. silvaticus Schaeff ex. Secr* substrate [2].In 2011, Ueguchi *et al*. found N-benzoyl-L-leucine methyl ester from *A. blazei* substrate, which is the first time that this compound was isolated from nature [4].

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4. Aliphatic compounds

Aliphatic compounds, also known as open-chain compounds, are molecules in which the carbon atoms are connected in chains, including alkanes, alkenes, alkynes and their derivatives. Mushrooms have an aroma and the main reason for this is the presence of volatile aliphatic compounds in mushrooms.

In 2016, Ragasa *et al*. isolated trilinolein and linoleic acid from *A. blazei* subspecies [13], trilinolein has been reported to have a protective effect against cardiovascular diseases [21], and linoleic acid reduces the risk of colon and breast cancers [22]. 2018. Waqas *et al.* identified pentadecanoic acid, 14-methyl-, methyl ester, 1-hexadecanol, 2-methyl-, 9, 12-octadecadienoic acid (*Z*, *Z*)-, methyl ester from *A. blazei* substrates by GCMS [23]. In 2021, Pham *et al*. isolated (9*Z*,12*Z*)-9,12-octadecanoic acid from *A. bisporus* substrates in their search for anti-peroxisome proliferator-activated receptor agonists (PPARs) [24].In 2022, Wang *et al*. isolate five fatty acid compounds from *Agaricus blazei*, all of which showed inhibitory effects against the phytopathogenic bacterium, Lactobacillus michiganensis [25].

5. Other compounds

In 2005, Stadler *et al*. obtained nine agaric acid glycerides from *A. macrosporus* mycelium, namely agaricoglyceride A, agaricoglyceride B, agaricoglyceride C, agaricoglyceride D, (3,5-dichloro-4-methoxyphenyl)methanol, 3,5-dichloro-4-anisic acid, agaric ester, monoacetyl-agaricoglycerides A (separated into inseparable mixtures), and all compounds had a positive effect on neurolysin and angiotensin-converting enzyme, with IC_{50}= 0.2 μmol/L for agaricoglyceride A and 0.05 μmol/L for monoacetyl-agaricoglycerides in the anti-neurolysin [26].In 2008, Barros *et al*. used normal-phase high performance liquid chromatography (HPLC) to determine the content of tocopherol in the ascospores of *A. arvensis*, *A. bisporus*, *A. romagnesii*, *A. silvaticus* and *A. silvicola*, and the results showed that all five species of fungi contained α-tocopherol, and except *A. romagnesii*, four fungi contained β-tocopherol [27].In 2011, Ueguchi *et al*. identified two phenylhexane derivatives from *A. blazei* substrates, 1-[4-(Hydroxymethyl)phenyl]hexan-1-ol and 4′-hydroxymethyl-1-phenyl-n-hexan-1-one, both of them showed moderate cytotoxicity against HepG2 cells with IC_{50} 39.2 ± 5.68 μmol/L and 28.9 ± 2.06 μmol/L, respectively [11]. In 2013, Dong *et al*. obtained brefeldin A, an active component of Erk1/2 with oestrogenic activity from *A. blazei* mycelium [28].In 2016, Hammann *et al*. identified an ester, drimenol linoleic acid ester, from *A. blazei* substrates using GCMS [29].In 2018, Waqas identified seven compounds from *A. blazei* substrates by GCMS, such as 1,2- benzenedicarboxylic acid, mono (2-ethylhexyl) ester *et al*. and 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester inhibited Streptomyces intermedius and Fusarium spinosum by 44% and 50% [23].In 2020, Wu *et al*. obtained fourteen other types of compounds from *A. gennadii*, such as macrosphelide A, 7-acetyl-4-methyl-1-azulenecarboxylic acid *et al*. [13].

6. Discussion and outlook

At present, the study of active secondary metabolites of fungi of the genus Mushroom has been more in-depth, and the secondary metabolites that have been identified are chemically diverse, have novel skeletons, and are rich in biological activities. Studies have shown that Mushroom spp. contain various types of compounds such as steroids, terpenoids, nitrogenous compounds, sphingosine esters, aliphatic compounds, etc., which show a variety of strong biological activities such as anti-tumour, anti-bacterial, anti-neurolysin, anti-angiotensin converting enzyme, and so on. Therefore, the rich source of Mushroom fungi has a great potential for both food and new drug development and utilisation. Through literature review, it is found that the current research on mushroom fungi mainly focuses on one species, *A. blazei*, and the composition of other fungi in this genus needs further in-depth study, which is of high value for the development and utilisation of active novel structural compounds. Although the secondary metabolites of *A. blazei* have been studied, most of the bioactivities of *A. blazei* have been investigated in the extracts of fruiting bodies or mycelium, and the bioactivities of single compounds have been less studied, so in-depth studies on the bioactivities of single compounds in this genus can be a direction for future research and provide lead compounds for drug development.

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7. Conclusion

Through the exploration of the secondary metabolites of Tricholoma matsutake fungus, we have discovered a plethora of bioactive compounds. Among them, steroids and terpenoids are particularly abundant in the secondary metabolites of matsutake, exhibiting antibacterial, anti-inflammatory, and antioxidant properties that hold significant importance for human health. Furthermore, nitrogen-containing compounds play a pivotal role in the secondary metabolites of matsutake. Nitrogenous heterocycles possess antimicrobial and antitumor effects, while amino acid compounds enhance immune function and contribute to growth and development. Phospholipids play a crucial role in cellular signal transduction and immune regulation. The bioactivities of other nitrogen-containing compounds require further exploration and elucidation. Additionally, the secondary metabolites of matsutake consist of a rich array of fatty compounds, which have substantial regulatory effects on the cardiovascular, nervous, and immune systems, playing a vital role in human health. In addition to the aforementioned constituents, the secondary metabolites of matsutake also contain other compounds, such as polysaccharides, polyphenols, and vitamins, which contribute to immune regulation, antioxidant activity, and anti-tumor properties within the body. To summarize, the secondary metabolites of Tricholoma matsutake fungus encompass a diverse range of compounds with various bioactivities, holding immense significance for human health. However, our current understanding of matsutake secondary metabolites is still in its nascent stages, with numerous uncharted areas awaiting further exploration. Future research endeavors can delve into elucidating their mechanisms of action, optimizing extraction processes, and exploring their applications in the fields of food, medicine, and health supplements. With an increased understanding of matsutake secondary metabolites, we believe they will bring more well-being to human health.

References

[1] Petrova A, et al. GC-MS studies of the chemical composition of two inedible mushrooms of the genus Agaricus[J]. CHEM CENT J, 2007, 1(1): 33.
[2] Munkhgerel L, et al. Chemical and biological investigation of the Agaricus silvaticus Schaeff ex. Secr[J]. Mongolian Journal of Chemistry, 2011, 12: 92-97.
[3] Shin Y, et al. Chemical Constituents of Inonotus ohliquus[J]. Eurasian J. For. Res., 2001, 2: 27-30.
[4] Ueguchi Y, et al. Constituents of cultivated Agaricus blazei[J]. J Nat Med, 2011, 65(2): 307-312.
[5] Shimizu T, et al. Agarol, an ergosterol derivative from Agaricus blazei, induces caspase-independent apoptosis in human cancer cells[J]. Int J Oncol, 2016, 48(4): 1670-1678.
[6] Ragasa C Y, et al. Sterols and Lipids from Agaricus bisporus[J]. Int. J. Pharm. Clin. Res., 2016, 8(10): 1451-1453.
[7] Sun Y, et al. Sterols from Mytilidae show anti-aging and neuroprotective effects via anti-oxidative activity. Int J Mol Sci, 2014, 15(12):21660-21673.
[8] Misgiati M, Aty W,Sentot J R, et al. Ergosterol isolated from Agaricus blazei Murill N-hexane extracts as potential anticancer MCF-7 activity[J]. Pharmacogn J. 2021; 13(2): 418-426.
[9] Hirotani M, et al. Blazeispirane and protoblazeispirane derivatives from the cultured mycelia of the fungus Agaricus blazei[J]. Phytochemistry, 2002, 61(5): 589-595.
[10] Hirotani M, et al. Agariblazeispirols A and B, an unprecedented skeleton from the cultured mycelia of the fungus, Agaricus blazei[J]. Tetrahedron Letters, 2003, 44(43): 7975-7979.
[11] Hirotani M, et al. Agariblazeispirol C from the cultured mycelia of the fungus, Agaricus blazei, and the chemical conversion of blazeispirol A[J]. Tetrahedron, 2005, 61(1): 189-194.
[12] Itoh H, et al. Blazein of a new steroid isolated from Agaricus blazei Murrill (himematsutake) induces cell death and morphological change indicative of apoptotic chromatin condensation in human lung cancer LU99 and stomach cancer KATO III cells[J]. ONCOL REP, 2008, 20(6): 1359-1361.
[13] Wu J, et al. Chemical constituents of Agaricus gennadii[J]. CHEM NAT COMPD+, 2020, 56(4): 761-762.
[14] Zhao J Y, et al. Two new sesquiterpenes from cultures of the basidiomycete Agaricus arvensis[J]. J Asian Nat Prod Res, 2013, 15(3): 305-309.
[15] Kimura Y, et al. Isolation of an anti-angiogenic substance from Agaricus blazei Murill: Its antitumor and antimetastatic actions[J]. Cancer Science, 2004, 95(9): 758-764.
[16] Kohno K, et al. Anti-inflammatory and immunomodulatory properties of 2-amino-3H-phenoxazin-3-one[J]. BIOL PHARM BULL, 2008, 31(10): 1938-1945.
[17] Tian Y, Gui W, Smith P B, et al. Isolation and Identification of Aryl Hydrocarbon Receptor

ISSN 2616-5910 Vol. 4, Issue 1: 62-66, DOI: 10.25236/AJALS.2023.040109

Modulators in White Button Mushrooms (Agaricus bisporus) [J]. J Agric Food Chem, 2019, 67(33): 9286-9294.
[18] Janák K, Størmer F C, Koller G E. The content of agaritine in spores from Agaricus bisporus[J]. Food Chemistry, 2006, 99(3): 521-524.
[19]Jennemann R, et al. Novel glycoinositolphosphosphingolipids, basidiolipids, from Agaricus[J]. Eur J Biochem, 1999, 259(1-2): 331-338.
[20] Berg A, et al. Agaridiol, a new 1-arylpropane-1, 2-diol produced by Agaricus sp[J]. J BASIC MICROB, 1999, 39(3), 213-215.
[21] Chan P, et al. Protective effects of trilinolein extracted from Panax notoginseng against cardiovascular disease[J]. Acta Pharmacol Sin, 2002, 23(12):1157-1162.
[22]Sun Y, et al. Sterols from Mytilidae show anti-aging and neuroprotective effects via anti-oxidative activity[J]. Int J Mol Sci, 2014, 15(12):21660–21673.
[23] Waqas H M, Akbar M, Khalil T, et al. Identification of natural antifungal constituents from Agaricus bisporus (J. E. Lange) imbach[J]. Applied Ecology and Environmental Research, 2018, 16(6): 7937-7951.
[24] Pham N L,, et al. Effect-directed analysis and chemical identification of agonists of peroxisome proliferator-activated receptors in white button mushroom[J]. Food and Function, 2021, 12(1),133-134..
[25] Wang J H, et al. Anti-phytopathogenic-bacterial fatty acids from the mycelia of the edible mushroom Agaricus blazei[J]. Biosci Biotechnol Biochem, 2022, 86(10):1327-1332.
[26] Stadler M, et al. Novel analgesic triglycerides from cultures of Agaricus macrosporus and other basidiomycetes as selective inhibitors of neurolysin[J]. J ANTIBIOT, 2005, 58(12): 775-786.
[27] Barros L, et al. Optimization of the determination of tocopherols in Agaricus sp. edible mushrooms by a normal phase liquid chromatographic method[J]. Food Chemistry, 2008, 110(4): 1046-1050.
[28] Dong S, et al. Brefeldin A is an estrogenic, Erk1/2-activating component in the extract of Agaricus blazei mycelia[J]. J AGR FOOD CHEM, 2013, 61(1): 128-136.
[29] Hammann S, Vetter W. Method development for the determination of free and esterified sterols in button mushrooms (Agaricus bisporus) [J]. J AGR FOOD CHEM, 2016, 64(17): 3437-3444.