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An *in vitro* evaluation of the cytotoxic potential of medicinal mushrooms against human breast cancer cell lines

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Medicinal mushroom extracts, i.e. their dried biomass, have long been known as sources of bioactive compounds with positive effects on the human health. The antioxidant, antigenotoxic, antiviral, and immunomodulatory properties of the commercially available extracts *Agaricus blazei* auct. non Murrill (AB), *Cordyceps sinensis* (Berk.) Sacc. (CS), and Immune Assist (IA) have already been documented. This study, studied the influence of these three mushrooms on the viability of cell lines MCF-7, MDA-MB-231, and HS-5. The cytotoxicity of AB, CS, and IA at different concentrations (25, 50, 100, 200, 400 and 800 μg/mL) was evaluated using the MTT assay. The results showed that AB was the most effective and induced cytotoxicity in both cancer cell lines, with IC_{50} values of 96.7 μg/mL for MCF-7 and 368.4 μg/mL for MDA-MB-231. After treatment with CS and IA, the half-maximal inhibitory concentration was reached only in MDA-MB-231 cells (IC_{50}=613 μg/mL for CS and 343.3 μg/mL for IA). We have shown here that AB, CS and IA can suppress the growth of MCF-7 and MDA-MB-231 cell lines, while affecting the survival of healthy HS-5 cells to a much lesser extent. Our *in vitro* results suggested that AB, CS and IA are promising natural sources with potential anticancer activity.

KEY WORDS: cytotoxicity; *Agaricus blazei; Cordyceps sinensis*; Immune Assist; MTT

Mushroom extracts, which includes all stages of their development, are used as food supplements in the form of capsules or tablets with numerous nutritional values and account for around 10 % of the total food supplement market. Nearly 1 % of mushroom species are used for therapeutic purposes; however, thus far they have been neglected in research even though they possess a potential that deserves to be explored (1). The biological activities and pharmacological properties of known traditional medicinal mushrooms have already been documented. These include antiviral, antioxidant, antibacterial, antihypertensive, anticancer, anti-inflammatory, immunostimulant, antidiabetic, and anti-allergic effects (2–7).

Cancer is one of the most feared diseases worldwide and the leading cause of death in the 21st century. Breast cancer is the most common and still the most invasive form of cancer in women (8). While early identification and accurate diagnosis are essential in cancer care, recent studies have concentrated on creating new cancer treatments that utilize a non-toxic therapeutic approach. More than 60 % of cancer drugs can be traced back to a natural product, but none have yet been derived from a mushroom (9). This is surprising, as the mushroom species of the genera *Agaricus*, *Cordyceps*, *Ganoderma*, *Trametes*, etc. have long been known as sources of bioactive compounds that benefit human health, particularly for their potential roles in cancer prevention and therapy in traditional medicine (10–13).

We therefore aimed to evaluate the efficacy of the commercially available *Agaricus blazei* auct. non Murrill (AB), *Cordyceps sinensis* (Berk.) Sacc. (CS), and Immune Assist (IA) in the treatment of breast cancer cells *in vitro*. Although their antioxidant, antigenotoxic, antiviral, immunomodulatory, and anticancer properties have been previously reported (11, 12, 14–16), their effects on breast cancer cell lines have not been sufficiently explored. In this work, we investigated whether AB, CS, and IA can indeed also influence the proliferation/viability of breast cancer MCF-7 and MDA-MB-231 cell lines and the non-transformed bone marrow stromal HS-5 cells.

MATERIALS AND METHODS

Mushroom extracts

Commercial products of AB, CS, and IA in the form of powder capsules with known composition (14–16) were supplied by Aloha

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Medicinals Inc (Santa Cruz, CA, USA). While AB and CS are powders from one species of medicinal mushroom, IA is a blend of six species: *Agaricus blazei*, *Cordyceps sinensis*, *Grifola frondosa*, *Ganoderma lucidum*, *Trametes versicolor*, and *Lentinula edodes*. For the experiments, stock solutions of AB, CS, and IA were prepared at a concentration of 40 mg/mL in 50 % dimethyl sulfoxide (DMSO, Fisher Scientific, Pittsburgh, PA, USA) and 50 % phosphate-buffered saline (PBS, Fisher Scientific, Pittsburgh, PA, USA) from which series of final concentrations of 25, 50, 100, 200, 400, and 800 μg/mL were diluted in Eppendorf tubes in growth medium, GM. Control cells were exposed to an equivalent concentration of DMSO not exceeding 1 % at the highest treatment concentration. The range of final concentrations was chosen based on results from our previous studies on medicinal mushrooms (14, 15).

Cell culture

Human bone marrow stromal cells HS-5 (ATCC® CRL-11882™), human breast adenocarcinoma MCF-7 (ATCC® HTB-22™), and human breast adenocarcinoma triple-negative MDA-MB-231 (ATCC® HTB-26™) cell lines were maintained in Ham's F12: DMEM (1:1) (growth medium, GM) (Sigma Chemicals Co, St. Louis, MI, USA) augmented with 10 % heat-inactivated fetal bovine serum (PAA GmbH, Pasching, Austria). The cell culture media were enriched with streptomycin (200 μg/mL), penicillin (100 U/mL), L-glutamine (2 mM), and HEPES (10 mM). Cells were grown at 37 °C in an environment with 5 % CO_2 and humidified air.

MTT assay

The antiproliferative/cytotoxic effects of CS, AB and IA were evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 5×10^3 cells/well was seeded in 96-well plates in GM. On the following day, the medium was removed and fresh GM containing the test substances (in a concentration range of 25 to 800 μg/mL) was added to each well in a total culture volume of 100 μL, and the cells were incubated for a further 3 days. In parallel, the cells were treated with the same amounts of DMSO to determine the toxicity of the solvent. MTT was introduced into each well at a concentration of 0.5 mg/mL, and the cells were incubated for two hours. The culture medium was removed, and the formazan crystals that formed from the cells were dissolved in isopropanol:DMSO (3:2). The absorbance was measured at 630 nm (Victor X2 Multilabel Microplate Reader, PerkinElmer, Waltham, MA, USA). Each experiment was performed in triplicate. The results are presented as percentage over control values obtained for untreated cells. The proliferation curves were plotted using Excel software, and 50 % inhibitory concentration (IC_{50}), as the concentration of drug required for 50 % inhibition of cell proliferation (17), was calculated using an online calculator (18).

Statistical analysis

All experiments were performed in triplicate, and the results are presented as means ± standard error of the mean (mean±SEM). Statistical significance was assessed using Student's t-test. The level of significance was set to <0.05.

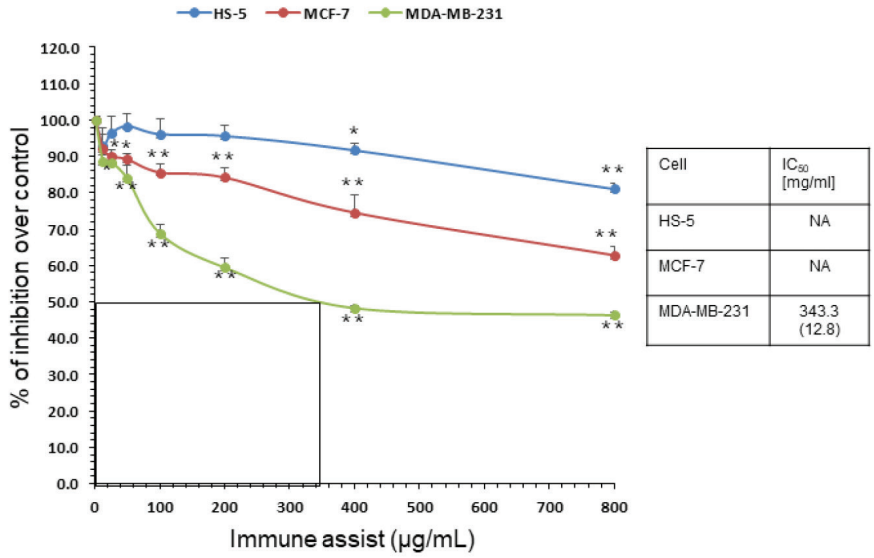
RESULTS AND DISCUSSION

The aim of the present study was to demonstrate the effect of AB, CS, and IA in the treatment of human breast cancer cells (MCF-7 and MDA-MB-231) and bone marrow stromal cells (HS-5) *in vitro*. MCF-7 cells have the tumor suppressor gene p53 as wild type, while MDA-MB-231 cells have p53 mutations (19). In addition, MCF-7 cells are estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) receptor positive and are classified as luminal type (low grade). MDA-MB-231 cells, on the other hand, show negative expression of ER, PR and HER2 [triple negative breast cancer (TNBC)] and are classified as basal type (high grade) (20, 21). Although TNBC accounts for only 15–20 % of all breast cancers, there are limited treatment options for this type of cancer as these cells do not respond to hormones or targeted therapies (22). Given the lack of validated molecular targets and the poor treatment outcomes in patients with TNBC, there is a clear need to develop better therapies. For this reason, it is of great importance to find agents that can act on these highly metastatic cells.

The results obtained in this study show that all three extracts reduce the viability of MDA-MB-231 cells. Figures 1, 2, and 3 show the effects of the concentration ranges (25, 50, 100, 200, 400, and 800 μg/mL) of AB, CS, and IA on the proliferation/viability of the MCF-7, MDA-MB-231, and HS-5 cell lines.

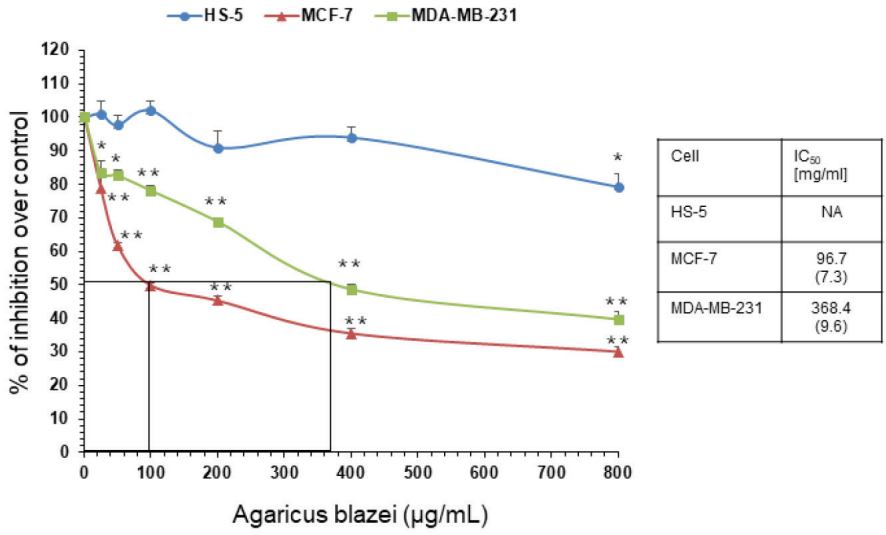
IA had the best effect on MDA-MB-231 cells with an IC_{50}=343.3 μg/mL (Figure 1). This corresponds to a 7 % higher efficacy than AB (IC_{50}=368.4 μg/mL) (Figure 2) and 78 % higher than CS (IC_{50}=613 μg/mL) (Figure 3). Treatment of the second breast cancer cell line (MCF-7) with IA for 72 hours resulted in some degree of cytotoxic effect, but the IC_{50} was not reached at any of the concentrations tested. In addition, the cytotoxic effect of AB was demonstrated both in MDA-MB-231 and MCF-7 cells (IC_{50} of 96.7 μg/mL for MCF-7). Moreover, CS reached its 50 % inhibitory concentration only in MDA-MB-231 cells. The most potent of the three compounds was AB, reaching the IC_{50} in both cancer cell lines, with the treatment having the strongest overall effect on MCF-7 cells. Toxicity to healthy cells (HS-5) was significantly lower for all treatments and did not reach the IC_{50} at any of the concentrations tested. These data are noteworthy as they indicate that the extracts are more toxic to cancer cells than to healthy cells.

It has been documented that exposure of the MCF-7 and MDA-MB-231 cell lines to cordycepin (3-deoxyadenosine) from CS resulted in a dose-dependent inhibition of cell growth and reduced cell viability (23). Research into the pharmacological effects of



Cell	IC_{50} [mg/ml]
HS-5	NA
MCF-7	NA
MDA-MB-231	343.3 (12.8)

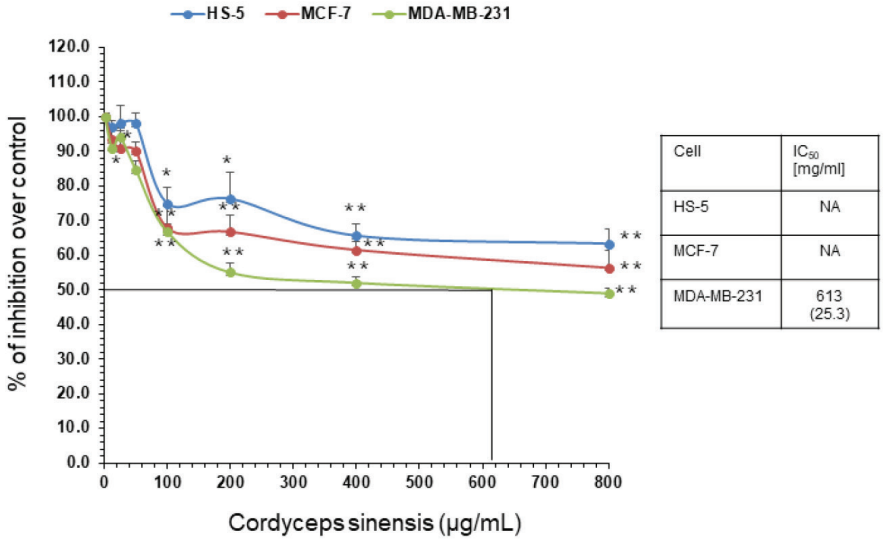
Figure 1 Evaluation of the cytotoxic effects of Immune Assist on HS-5, MCF-7, and MDA-MB-231 cell lines using MTT assay measured after 72 hours treatment. Results are shown as mean±SEM. Statistical significance was assessed using Student's t-test. *p<0.05,**p<0.005 vs. control



Cell	IC_{50} [mg/ml]
HS-5	NA
MCF-7	96.7 (7.3)
MDA-MB-231	368.4 (9.6)

Figure 2 Evaluation of the cytotoxic effects of *Agaricus blazei* on HS-5, MCF-7, and MDA-MB-231 cells using MTT assay measured after 72 hours treatment. Results are shown as mean±SEM. Statistical significance was assessed using Student's t-test. *p<0.05,**p<0.005 vs. control

mushroom extracts that are components of IA (*A. blazei*, *C. sinensis*, *G. frondosa*, *G. lucidum*, *L. edodes*, and *T. versicolor*) and their bioactive compounds in cancer treatment and prevention has already shown that they have suppressive activity against MCF-7 and MDA-MB-231 cells through various forms of cytotoxic effects. An aqueous extract from the fruiting body of *Lentinula edodes* showed significant dose-dependent inhibitory effects on the proliferation of MCF-7 cells (24). The viability and cell migration of MDA-MB-231 cells were impaired by an extract from *Ganoderma lucidum* (25), while oral administration of the extract for one month decreased the expression of genes involved in the invasive behavior of MDA-MB-231 cells and prevented their migration (26). In addition, the ethanol extract of *Trametes versicolor* suppressed the proliferation of the MCF-7 and MDA-MB-231 breast tumor cell lines in a dose-dependent manner (27). In turn, MycoPhyto® Complex, a mixture of fungal mycelia from *Cordyceps sinensis*, *Coriolus versicolor*, *Ganoderma lucidum*, *Grifola frondosa*, *Agaricus blazei* and *Polyporus umbellatus* as well as β-1,3-glucan from yeast, suppressed the proliferation of MDA-MB-231 in a dose- (0–0.5 mg/mL) and time-dependent (24, 48, 72 h) manner and arrested the cells in the G2/M phase of the cell cycle (28). Moreover, treatment with polysaccharides from *Grifola frondosa* increased the accumulation of reactive oxygen species and induced mitochondrial dysfunction in MCF-7 and MDA-MB-231 breast



Cell	IC_{50} [mg/ml]
HS-5	NA
MCF-7	NA
MDA-MB-231	613 (25.3)

Figure 3 Evaluation of the cytotoxic effects of *Cordyceps sinensis* on HS-5, MCF-7, and MDA-MB-231 cell lines using MTT assay measured after 72 hours treatment. Results are shown as mean±SEM. Statistical significance was assessed using Student's t-test. *p<0.05,**p<0.005 vs. control

cancer cells (29). Ganodermanontriol from *G. lucidum* inhibited the proliferation and colony formation of MDA-MB-231 cells (30).

A mushroom extract that is successful against cancer should be able to eliminate cancer cells while protecting healthy cells, such as HS-5, from excessive damage. Previously, latcripin-7A extracted from *Lentinula edodes* was shown to induce cell cycle arrest and promote autophagy, leading to apoptotic cell death in MCF-7 and MDA-MB-231 cells without affecting the survival of healthy breast MCF-10A cells (31). Our results are consistent with this and show that 72-hour incubation with different concentrations of AB, CS and IA (25–800 µg/mL) induces some degree of cytotoxicity in HS-5 cells, but does not reach the half-maximal inhibitory concentration. The MTT assay as a simplified 2D standard model for cytotoxicity provides a basis and insight into possible future investigations of the effects of the tested substances. Due to the method used, we cannot speculate on the mechanisms and reasons for the effects obtained based on our results. However, based on the above-mentioned *in vitro* and *in vivo* studies of medicinal mushrooms and their constituents, it is plausible to assume that the effect of some active ingredients in AB, CS and IA from groups of polysaccharides, polyphenols, sterols or catechols may influence the overall outcome. Studies have shown to some extent that the anticancer effects may be due to their antioxidant and free radical scavenging activity, inhibition of metabolic activation and enhancement of detoxification of carcinogens, direct cytotoxicity, antiproliferation and modulation of signal transduction molecules, and induction of cell cycle arrest and apoptosis (11, 12, 23, 28–31). This preliminary study provides evidence for further analysis of the effects of AB, CS and IA, their underlying mechanisms and the possibility of evaluating combined therapies with conventional treatments.

CONCLUSION

Our study has shown that AB, CS and IA can suppress the growth of highly metastatic MDA-MB-231 cells. AB proved to be the most potent and cytotoxic extract for MCF-7 and MDA-MB-231 cell lines. To our knowledge, this is the first study to show that traditional medicinal mushrooms can suppress the growth of human breast cancer cell lines *in vitro*, while affecting the survival of healthy cells to a much lesser extent. This study provides an initial structure for future research to determine the exact molecular mechanism responsible for the anticancer effect of AB, CS, and IA. In addition, AB, CS, and IA should be further investigated *in vivo* for use in combination with chemotherapeutic agents as adjuvants in breast cancer therapy.

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Conflict of interest

None to declare.

REFERENCES

1. Panda SK, Sahoo G, Swain SS, Luyten W. Anticancer activities of mushrooms: a neglected source for drug discovery. Pharmaceuticals 2022;15(2):176. doi: 10.3390/ph15020176
2. Kosanić M, Ranković B, Rančić A, Stanojković T. Evaluation of metal concentration and antioxidant, antimicrobial, and anticancer potentials of two edible mushrooms *Lactarius deliciosus* and *Macrolepiota procera*. J Food Drug Anal 2016;24:477–84. doi: 10.1016/j.jfda.2016.01.008
3. Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, Martínez JA, García-Lafuente A. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem 2012;130:350–5. doi: 10.1016/j.foodchem.2011.07.049
4. Nowakowski P, Naliwajko SK, Markiewicz-Żukowska R, Borawska MH, Socha K. The two faces of *Coprinus comatus*—Functional properties and potential hazards. Phyther Res 2020;34:2932–44. doi: 10.1002/ptr.6741
5. Ramesh Ch, Pattar MG. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India. Pharmacognosy Res 2010;2:107-12. doi: 10.4103/0974-8490.62953
6. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Appl Microbiol Biotechnol 2011;89:1323–32. doi: 10.1007/s00253-010-3067-4
7. Chang ST, Wasser SP. The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. Int J Med Mushrooms 2012;14:95–134. doi: 10.1615/intjmedmushr.v14.i2.10
8. Luis C, Castaño-Guerrero Y, Soares R, Sales G, Fernandes R. Avoiding the interference of Doxorubicin with MTT measurements on the MCF-7 breast cancer cell line. Methods Protoc 2019;2(2):29. doi: 10.3390/mps2020029
9. Demain AL, Vaishnav P. Natural products for cancer chemotherapy. Microb Biotechnol 2011;4:687–99. doi: 10.1111/j.1751-7915.2010.00221.x
10. Hahne JC, Meyer SR, Dietl J, Honig A. The effect of *Cordyceps* extract and a mixture of *Ganoderma lucidum*/*Agaricus Blazei Murill* extract on human endometrial cancer cell lines *in vitro*. Int J Oncol 2014;45:373–82. doi: 10.3892/ijo.2014.2414
11. Figueiredo L, Régis WCB. Medicinal mushrooms in adjuvant cancer therapies: an approach to anticancer effects and presumed mechanisms of action. Nutrire 2017;42(1):28. doi: 10.1186/s41110-017-0050-1
12. Nandi S, Sikder R, Rapior S, Arnould S, Simal-Gandara J, Acharya K. A review for cancer treatment with mushroom metabolites through targeting mitochondrial signaling pathway: *In vitro* and *in vivo* evaluations, clinical studies and future prospects for mycomedicine. Fitoterapia 2024;172:105681. doi: 10.1016/j.fitote.2023.105681
13. Park H-J. Current uses of mushrooms in cancer treatment and their anticancer mechanisms. Int J Mol Sci 2022;23(18):10502. doi: 10.3390/ijms231810502
14. Živković L, Borozan S, Čabarkapa A, Topalović D, Ciptasari U, Bajić V, Spremo-Potparević B. Antigenotoxic properties of *Agaricus blazei* against hydrogen peroxide in human peripheral blood cells. Oxid Med Cell Longev 2017;2017:8759764. doi: 10.1155/2017/8759764
15. Živković L, Borozan S, Bajić V, Đorđević S, Hristov A, Spremo-Potparević B. Evaluacija antioksidativnog potencijala gljive *Cordyceps sinensis in vitro* [Evaluation of antioxidant potential of *Cordyceps sinensis in vitro*, in Serbian]. Med Čas (Krag) 2019;53:129–34.
16. Adotey G, Quarcoo A, Holliday JC, Fofie S, Saaka B. Effect of immunomodulating and antiviral agent of medicinal mushrooms (immune assist 24/7™) on CD4+ T-lymphocyte counts of HIV-infected patients. Int J Med Mushrooms 2011;13:109–13. doi: 10.1615/IntJMedMushr.v13.i2.20
17. Swinney DC. Chapter 18 – Molecular mechanism of action (MMoA) in drug discovery. Ann Rep Med Chem. 2011;46:301–17. doi: 10.1016/B978-0-12-386009-5.00009-6
18. AAT Bioquest. EC50 calculator [displayed 13 December 2024]. Available at https://www.aatbio.com/tools/ec50-calculator
19. Lacroix M, Toillon R-A, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer 2006;13:293–325. doi: 10.1677/erc.1.01172
20. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res 2020;22(1):61. doi: 10.1186/s13058-020-01296-5
21. Kenny PA, Lee GY, Myers CA, Neve RM, Semeiks JR, Spellman PT, Lorenz K, Lee EH, Barcellos-Hoff MH, Petersen OW, Gray JW, Bissell MJ. The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression. Mol Oncol 2007;1:84–96. doi: 10.1016/j.molonc.2007.02.004
22. Wei C, Khan MA, Du J, Cheng J, Tania M, Leung ELH, Fu J. Cordycepin inhibits triple-negative breast cancer cell migration and invasion by regulating EMT-TFs SLUG, TWIST1, SNAIL1, and ZEB1. Front Oncol 2022;12:898583. doi: 10.3389/fonc.2022.898583
23. Choi S, Lim MH, Kim KM, Jeon BH, Song WO, Kim TW. Cordycepin-induced apoptosis and autophagy in breast cancer cells are independent of the estrogen receptor. Toxicol Appl Pharmacol 2011;257:165–73. doi: 10.1016/j.taap.2011.08.030
24. Israilides C, Kletsas D, Arapoglou D, Philippoussis A, Pratsinis H, Ebringerová A, Hríbalová V, Harding SE. *In vitro* cytostatic and immunomodulatory properties of the medicinal mushroom *Lentinula edodes*. Phytomedicine 2008;15:512–9. doi: 10.1016/j.phymed.2007.11.029
25. Barbieri A, Quagliariello V, Del Vecchio V, Falco M, Luciano A, Amruthraj N, Nasti G, Ottaiano A, Berretta M, Iaffaioli RV, Arra C. Anticancer and anti-inflammatory properties of *Ganoderma lucidum* extract effects on melanoma and triple-negative breast cancer treatment. Nutrients 2017;9(3):210. doi: 10.3390/nu9030210
26. Loganathan J, Jiang J, Smith A, Jedinak A, Thyagarajan-Sahu A, Sandusky GE, Nakshatri H, Sliva D. The mushroom *Ganoderma lucidum* suppresses breast-to-lung cancer metastasis through the inhibition of pro-invasive genes. Int J Oncol 2014;44:2009–15. doi: 10.3892/ijo.2014.2375
27. Ho CY, Kim CF, Leung KN, Kwok-Pui F, Tse TF, Chan H, Lau CB. Differential anti-tumor activity of coriolus versicolor (Yunzhi) extract through p53- and/or Bcl-2-dependent apoptotic pathway in human breast cancer cells. Cancer Biol Ther 2005;4:638–44. doi: 10.4161/cbt.4.6.1721
28. Jiang J, Sliva D. Novel medicinal mushroom blend suppresses growth and invasiveness of human breast cancer cells. Int J Oncol 2010;37:1529-36. doi: 10.3892/ijo_00000806
29. Zhang Y, Sun D, Meng Q, Guo W, Chen Q, Zhang Y. *Grifola frondosa* polysaccharides induce breast cancer cell apoptosis via the mitochondrial-dependent apoptotic pathway. Int J Mol Med 2017;40:1089–95. doi: 10.3892/ijmm.2017.3081
30. Jiang J, Jedinak A, Sliva D. Ganodermanontriol (GDNT) exerts its effect on growth and invasiveness of breast cancer cells through the down-regulation of CDC20 and uPA. Biochem Biophys Res Commun 2011;415:325–9. doi: 10.1016/j.bbrc.2011.10.05
31. Gariboldi MB, Marras E, Ferrario N, Vivona V, Prini P, Vignati F, Perletti G. Anti-cancer potential of edible/medicinal mushrooms in breast cancer. Int J Mol Sci 2023;24(12):10120. doi: 10.3390/ijms241210120

Ispitivanje citotoksičnog potencijala gljiva za medicinsku upotrebu *in vitro* u ljudskih staničnih linija raka dojke

Ekstrakti gljiva za medicinsku upotrebu, odnosno njihova osušena biomasa, odavno su poznati kao izvori bioaktivnih spojeva s pozitivnim učinkom na ljudsko zdravlje. Antioksidacijska, antigenotoksična, antivirusna i imunomodulirajuća svojstva komercijalno dostupnih ekstrakata *Agaricus blazei* auct. non Murrill (AB), *Cordyceps sinensis* (Berk.) Sacc. (CS) i Immune Assist (IA) već su dugo poznata. Ovim istraživanjem ispitan je učinak tih triju gljiva na održivost staničnih linija MCF-7, MDA-MB-231 i HS-5. Citotoksičnost AB, CS i IA u različitim koncentracijama (25, 50, 100, 200, 400 i 800 μg/mL) procijenjena je MTT testom. Rezultati su pokazali da je AB izazvao najučinkovitiju citotoksičnost u objema staničnim linijama raka, s IC_{50} vrijednostima od 96,7 μg/mL za MCF-7 i 368,4 μg/mL za MDA-MB-231. Nakon tretmana s CS i IA polumaksimalna inhibitorna koncentracija postignuta je samo u stanicama MDA-MB-231 (IC_{50}=613 μg/mL za CS i 343,3 μg/mL za IA). Ovo je istraživanje pokazalo da AB, CS i IA mogu donekle suzbiti rast staničnih linija raka bez utjecaja na preživljavanje normalnih HS-5 stanica. Naši rezultati sugeriraju da su AB, CS i IA obećavajući prirodni izvori s potencijalnim djelovanjem protiv raka.

KLJUČNE RIJEČI: citotoksičnost; *Agaricus blazei*; *Cordyceps sinensis*; Immune Assist; MTT