



OPINION

By Assoc. prof. Galina Radeva, PhD

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of PhD thesis of Alexander Nikolaev Dushkov for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, professional area 4.3 Biological sciences (Molecular Biology)

Author: Alexander Nikolaev Dushkov

Title: „ Natural Low-Toxic Bioactive Extracts from Bulgarian Mushrooms with a Focus on *Amanita muscaria* – Composition Analysis, Antitumor Activity, and Mechanism of Action.

Scientific supervisor: Prof. Iva Ugrinova, PhD

1.General presentation of the materials under the procedure

By Order No. 218-ОБ/20.12.2024 of the Director of the Institute of Molecular Biology (IMB), I have been appointed as a member of the scientific jury for the defense of a dissertation for the acquisition of the educational and scientific degree "Doctor" in the professional field 4.3. "Biological Sciences," doctoral program "Molecular Biology." The dissertation, titled "Natural Low-Toxic Bioactive Extracts from Bulgarian Mushrooms with a Focus on *Amanita muscaria* – Composition Analysis, Antitumor Activity, and Mechanism of Action" is authored by Alexander Dushkov, a full-time doctoral student, supervised by prof. Iva Ugrinova, PhD from IMB.

The set of materials submitted by Alexander Dushkov on an electronic medium complies with the requirements of the Academic Staff Development Act of the Republic of Bulgaria (ASDA) and the Regulations for the Development of the Academic Staff of the Bulgarian Academy of Sciences (BAS) and IMB. It includes all documents required for the procedure.

2.Brief Biographical Data for the Doctoral Candidate

Alexander Dushkov holds a Bachelor's degree in Molecular Biology (2019) and a Master's degree in Cell Biology and Pathology (2021) from the Faculty of Biology at Sofia University "St. Kliment Ohridski." While still a student, Dushkov began working at the Institute of Molecular Biology (IMB) in the "Chromatin Structure and Function" section as a biological technician (2018), later advancing to the position of biologist (2019–2021). During this time, he completed his master's thesis under the supervision of Prof. Dr. Iva Ugrinova. In 2022, he was enrolled as a full-time PhD student in the same research section.

3.Characteristics of the dissertation work

3.1.Relevance of the Dissertation Topic and Feasibility of the Set Tasks

The antitumor potential of secondary metabolites from wood-decaying fungi is an actively researched topic in the fields of medical mycology and pharmacology. It is well known that

certain species of wood-decaying fungi (e.g., *Ganoderma lucidum*, *Trametes versicolor*, *Inonotus obliquus*) contain bioactive compounds that show potential as natural antitumor agents. Following clinical studies to confirm their efficacy and safety, these compounds could be used either independently or as complementary therapy alongside conventional cancer treatment methods. Currently, mycotherapy is successfully applied in various European countries not only for the treatment of oncological diseases but also for other conditions such as diabetes and respiratory problems. However, scientific research in our country regarding the potential bioactive compounds extracted from the fruiting bodies of local wood-decaying fungi remains limited.

To contribute to the research on this intriguing and relevant topic, the aim of this dissertation is to investigate the antitumor effect of extracts obtained from different mushroom species found in Bulgaria. More specifically, the objective is to obtain ethanol and aqueous extracts from the fly agaric (*Amanita muscaria*), containing the biologically active compounds—ibotenic acid, muscidiol, and ergosterol—and to study their cytotoxic effects on a panel of cancer cell lines, as well as their mechanisms of action on cellular processes and cell division. In view of the above, I consider the topic of the dissertation to be relevant and scientifically significant. Five specific tasks have been set, which I believe are well-focused, and their completion will lead to the achievement of the research objective.

3.2. Knowing the problem

The literature review examines various types of human cancers, including lung, prostate, skin, and breast cancer. It analyzes the historical progress in the use of mycotherapy for treating these diseases. Five species of wood-decaying fungi and their pharmacological properties are described in detail. Special attention is given to the antitumor effects of bioactive compounds on cellular processes, including DNA damage, chromatin modifications, and cellular stress. The structure of the literature review and the analytical presentation of the information demonstrate that Alexander Dushkov has an excellent understanding of the current state of research on the topic.

3.3. Research methods

To achieve the set objectives, a combination of modern cell biology and chromatographic methods has been selected. The study employs cell culture and treatment techniques, immunofluorescence analyses, and chromatographic methods (HPLC, UHPLC-MS/MS, CZE/CCD) for the separation and purification of bioactive compounds (ibotenic acid, muscimol, and ergosterol) from ethanol extracts of the mushrooms, as well as microscopic methods. The methods used in the dissertation are well-aligned with the research tasks, ensuring their successful completion.

3.4. Evaluation of the dissertation work

Dushkov's dissertation is written on 117 pages and contains 47 figures, 4 table and 248 cited references.

The dissertation is structured according to the commonly accepted requirements for this type of academic work and includes the following sections: Introduction (2 pages), Literature Review (37 pages), Aim and Objectives (1 page), Materials and Methods (20 pages), Results and Discussion (36 pages), Conclusions (1 page), Contributions (1 page), and References. Additionally, a list of the doctoral candidate's scientific publications and participation in scientific forums is attached to the dissertation. The abstract is written in 47 pages and accurately reflects the content of the dissertation and the contributions of the conducted research. The dissertation's objective is convincingly justified in light of the literature review. To achieve this objective, five specific tasks have been formulated, all of which align with the research goal. Each task has been addressed through experimental work, with results that are clearly presented, well-illustrated, and thoroughly discussed in the context of existing scientific literature on the subject. At the end of the dissertation, six conclusions are drawn, and six contributions of scientific and methodological significance are formulated, accurately reflecting the main findings of the experimental research.

I cannot fail to mention in my review the excellent impression left on me by the dissertation. It is engaging to read, written in clear and precise scientific language, and stylistically well-crafted.

The most significant scientific results and contributions from the research in the dissertation can be summarized as follows:

A varying degree of cytotoxic effect of aqueous and ethanolic extracts from five local wood-inhabiting mushroom species (*Trametes versicolor*, *Lenzites betulina*, *Fomes fomentarius*, *Fomitopsis betulina*, and *Amanita muscaria*) has been established against a panel of cancer cell lines in vitro. A comparative assessment of their cytotoxicity revealed that the ethanolic extract of *A. muscaria* exhibited the highest cytotoxicity, even at low concentrations, against lung and prostate cancer cell lines.

It has been established that the ethanolic extract of *A. muscaria* induces the formation of cytoplasmic structures known as stress granules (SGs) in treated lung cancer cell lines and also reduces the number of cells in the S-phase. These studies suggest that other compounds present in the extract act as stress factors on the cell and influence its response to them.

On the topic of his dissertation, Alexander Dushkov has presented two scientific publications in open-access journals indexed in the Web of Science database, with quartiles Q1 and Q4. The total number of points for indicator G is 37 (with a required minimum of 30 points) according to the national requirements for the PhD degree. In both publications, Dushkov is the first author, demonstrating his leading role and contribution to the conducted research and its presentation. The doctoral candidate has presented his results at four national and three international scientific forums.

Conclusion:

Alexander Dushkov's dissertation presents results that contribute to the development of new anticancer drugs based on extracts from wood-inhabiting fungi. The study employs appropriate chromatographic methods for their separation and purification, as well as an

analysis of their mechanism of action at the cellular level. The doctoral candidate has mastered a wide range of experimental methods and demonstrates the ability to present his scientific research to the academic community, establishing himself as a highly qualified young scientist. The dissertation meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and its implementing regulations, including the internal regulations of IMB. All this gives me grounds to give a positive evaluation of the conducted research, whose results are thoroughly presented in the dissertation. I strongly recommend that the esteemed scientific jury award the educational and scientific degree of "Doctor" to Alexander Nikolaev Dushkov in the field of higher education 4. Natural Sciences, Mathematics, and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Molecular Biology."

12.03.2025

Signed by:

/Assoc. prof. G. Radeva)