

To the chairman of the scientific jury,
as defined by Order No RD-15-121
/19.07.2022
of the Director of IEMPAM, BAS

STATEMENT REVIEW

On: PhD thesis submitted for the awarding of educational and scientific degree „Doctor” (PhD), Higher education area: 4. Natural sciences, mathematics and informatics, Professional field: 4.3. Biological sciences, Scientific specialty „Biochemistry” with code **01.06.10**

Candidate: Angeliki Nikolaus Konstantinidou, PhD student in the Department of Pathology at the Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) of the Bulgarian Academy of Sciences (BAS)

Thesis topic: „Optimization of the production of biologically active compounds from bacteria and algae and evaluation of their potential activity as anticancer agents in *in vitro* experiments“

Supervisors: Prof. Dr. Svetlozara Petkova and Assoc. Prof. Dr. Lyudmila Kabaivanova

Prepared by: Assoc. Prof. Nina Dimitrova Tsvetkova, PhD, National Center of Infectious and Parasitic Diseases, Sofia

I declare that I have no common scientific publications or conflict of interests of another nature within the meaning of para 1, items 3 and 5 of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) with the doctoral student.

Relevance of the PhD thesis topic

The present PhD thesis is an in-depth study of the potential anticancer activity of some biologically active compounds produced by bacterial and algal strains. The bacterial strains *Pseudomonas aeruginosa* and *Rhodococcus wratislavensis*, producers of glycolipid biosurfactants, and the red microalgae strains of the genera *Porphyridium* and *Rhodella*, producing sulfated exopolysaccharides, were selected as the study object. The study of the cytotoxic potential of these representatives of new types of natural substances on some types of cancer cells is aimed at their application as an alternative to the conventional antitumor therapy to overcome its shortcomings and side effects.

An essential moment of the scientific research is the selection of a suitable matrix for the immobilization of the strains. The immobilization technology of microbial cells allows to eliminate a significant part of the disadvantages of the free cell systems, as well as to create conditions for repeated use, for increase and maintenance of cell viability and biosynthetic activity of the microbial strains-producers used.

The lower cytotoxicity of the bioproducts synthesized by the tested bacterial and algal strains to non-cancerous cells established in the process of the experimental activity is of extreme importance for application in anti-cancer therapies. All this shows that the topic chosen by the supervisors for the PhD thesis is relevant and significant.

Characteristics and evaluation of the PhD thesis

The PhD thesis has been developed in the necessary volume and corresponds to the Law on the development of academic staff in the Republic of Bulgaria and the Rules for its implementation in the IEMPAM of the BAS. It contains 95 standard pages. It is illustrated with 45 figures. The bibliography includes 124 literary sources. Over 58% (n=47) of the publications cited in the literature review are from the last 10 years. The structure of the dissertation is standard.

The literature review is written in a volume of 25 pages, based on the selected literary sources containing information on the problem related to the topic of the PhD thesis.

The goals are clearly formulated and 6 specific tasks are set for their realization.

In the section "Material and methods" a set of modern complementary research methods and techniques (biochemical, cell-cultural, immunological, immobilization of microbial cells, spectrometric, chemical, chromatographic, cytological, scanning electron and fluorescence microscopy, experiments *in vivo*), which the PhD student has mastered and applied, leading to the achievement of the set tasks is described in detail. Both the objects of the study and the experimental approaches have been well chosen.

The data from the experimental work performed are described in the "Results and Discussion" section and are illustrated by 37 figures. The results correspond to the tasks set in the PhD thesis. The PhD student's ability to use data from the literature review when discussing his own results is impressive. The research has potential practical implications in the ongoing search for new methods and adjunctive therapies to impact colon and cervical carcinoma.

The conclusions (13 items) are correctly formulated and reflect the fulfillment of the goal and the set tasks.

I accept the contributions formulated by the PhD student. I consider the following to be the most important:

The effectiveness of the cryogel matrices of polyethylene oxide, polyacrylamide and hydroxyethylcellulose in the immobilization of bacterial and algal cells as a universal tool for increasing the yield of glycolipid biosurfactants and algal heteropolysaccharides has been established;

The effect of the resulting trehalose lipid was shown to be the highest when treating the cell line with low-metastatic potential (MCF-7), followed by that on the cell line MDA-MB-231 with the highly metastatic potential, and a stronger effect was observed when the highly metastatic cell line was treated with rhamnolipids.

A 44% reduction in cell viability was achieved when the highly metastatic MDA-MB231 cell line was treated with a heteropolysaccharide isolated from the red microalga *Rhodella reticulata*.

The effect of the application of the tested polysaccharide samples from the red microalga *Porphyridium cruentum* on the viability and proliferation of the following cell lines was established: - HT-29 colon carcinoma cells (the lowest cell viability values reaching 46%); - HeLa cervical carcinoma cells at all concentrations tested; and up to 25% decrease in MCF-7 tumor cell viability was achieved.

The **abstract** structurally reflects the essence of the PhD thesis and meets the requirements of the Law on the development of academic staff in the Republic of Bulgaria and the Regulations for its application in IEMPAM of the BAS.

Assessment of the PhD student's publications and personal contribution

The PhD thesis results were published in 2 journal articles in issues that are referenced and indexed in the world database Scopus with a Q3 rank in terms of SJR and were also presented at 1 international scientific forum. The publication activity of the PhD student in connection with the PhD thesis is sufficient and meets the requirements for this scientific degree, according to the Rules of IEMPAM of the BAS for application of the Law on the development of academic staff in the Republic of Bulgaria and the terms and conditions for acquiring the educational and scientific degree "Doctor".

CONCLUSION

Extensive, well-planned and precisely executed research work has been carried out. The assigned tasks have been completed. Original results were obtained with an appropriately selected combination of methods and conclusions were drawn which complement the knowledge of the mechanism of action of new potential anti-tumor agents in the fight against cancer. The contributions are original and significant, and would be useful in the development of new therapeutic strategies in the treatment of colon and uterine cancer.

The presented material meets the requirements for the defense of a PhD thesis for awarding the educational and scientific degree "Doctor", according to the Law on the development of the academic staff in the Republic of Bulgaria, and the rules for its implementation in the IEMPAM of the BAS.

I confidently give my positive assessment and strongly suggest to the respected members of the scientific jury to vote for the award of the educational and scientific degree "Doctor" in the Professional field 4.3. Biological Sciences (Scientific specialty "Biochemistry" – code 01.06.10.) to Angeliki Nikolaus Konstantinidou.

September 12, 2022

Signature:.....


/ Assoc. prof. Nina Tsvetkova, PhD /