

To the Chairman of the Scientific Committee, defined

by order No. ПД-15-121 / 19.07.2022

of the Director of IEMPAM-BAS

REVIEW

from

**Prof. Dr. Ioannis Vassilios Papathanasiou, MD, Ph.D.**  
**Department of Kinesitherapy**  
**Faculty of Public Health "prof. Dr. Tzecomir Vodenicharov, DSc"**  
**Medical University of Sofia**

Вх. № 191.....  
Дата 02.09.2022.....

**Regarding:** acquisition of the educational and scientific degree of the philosophical doctor "Ph.D." in professional field 4.3. Natural sciences and scientific specialty Biochemistry, with code 01.06.10 at the Institute of Experimental Morphology Pathology and Anthropology with Museum (IEMPAM) of Bulgarian Academy of Sciences (BAS).

**Author:** Mag. pharm. Angeliki Nikolaos Konstantinidou, Ph.D candidate in self-preparation at the IEMPAM of the BAS.

**Title:** Optimization of the production of biologically active substances from bacteria and algae and evaluation of their potential action as antitumor agents in vitro experiments.

The presented set of documents is in accordance with the regulations for the terms and conditions for acquiring scientific degrees and occupying academic positions at IEMPAM-BAS.

No plagiarism or evidence of plagiarism was detected during the review process of the submitted dissertation.

#### **Brief biographical data and professional development of the doctoral student**

Mag.Pharm. Angeliki Nikolaos Konstantinidou was born in Athens, Greece in 1993. She completed her secondary education in the 3rd General Lyceum in the city of Komotini, in 2011. She graduated in January 2018, with a master's pharmacist, from the Faculty of Pharmacy at the Medical University of Plovdiv. In the period from 01.12.2018 to 31.01.2021, she worked as a pharmacist in a large pharmacy chain, and from April to October 2021, she was a sales representative of a Bulgarian pharmaceutical company. Mag. Pharm. Angeliki Konstantinidou is a regular member of the Bulgarian Pharmaceutical Union

(BPHU) since 2018. She speaks and writes English fluently as well as has advanced computer literacy.

### **Relevance and significance of the topic**

The dissertation of mag. pharm. Angeliki Konstantinidou is competently and comprehensively written. The dissertation, entitled "Optimization of the production of biologically active substances from bacteria and algae and evaluation of their potential action as antitumor agents in vitro experiments", is dedicated to a still insufficiently developed topic for modern biochemistry and biotechnologies.

Nowadays, in scientific research a global tendency has been observed, i.e., to be focused on the investigation of new biologically active substances and new technologies for their use in various sectors. They are being conducted with the hope that their biological and medical applications will be widely used in the coming decades. This largely justifies the serious efforts to find a solution to various medical problems. The introduction in practice of new therapeutic agents of biological origin, isolated from concrete types of bacteria and algae and their potential possibility to be used in the treatment of tumors is justified due to their lower toxicity, as well as their significantly greater safety when compared to synthetic pharmaceutical products. Of the biologically active components isolated from algae, the most important for biology and medicine are complex polysaccharides, as well as some pigments. Considering the effect of some substances isolated from bacteria or algae against cancer cells, compounds that exhibit cytotoxic activity show exceptional effects in chemotherapy only when their toxicity is higher for cancer cells than for non-cancer cells. This selectivity towards specific cancer cell lines is one of the requirements for the development of new drugs that have a reduced degree of side effects.

Despite the intensive trials conducted in this area recently, there are some issues regarding the mechanism of action of the various bioproducts of natural origin at the cellular level, which gives the Ph.D. candidate Angeliki Konstantinidou a serious reason to focus her scientific interests in research of new natural substances of bacterial and algal origin.

### **Structure, design and visualization of the dissertation thesis**

The dissertation has been written in a volume of 95 standard typewritten pages, as well as 42 figures were designed for its illustration. It is properly structured in the following main sections: introduction, literature review, aim and objectives, material and methods, results, conclusions, contributions, and literature. The bibliographic reference is up-to-date and covers 124 literary references, all of which are in Latin.

## **Literature review**

The literature review is properly structured, detailed, and comprehensively presents the role of biologically active substances of bacterial origin applicable in biomedicine to solve problems related to human health. The Ph.D. candidate pays special attention to biologically active substances synthesized by algae, as well as to the immobilization of microbial cells. The literature review shows that the Ph.D. candidate has thoroughly studied all the issues related to her dissertation thesis, incl. and the current studies published on the topic.

## **Aim and tasks**

The aim of the Ph.D. thesis is precisely and clearly formulated, i.e., to investigate new natural substances of bacterial and algal origin, as well as to optimize their production and to test the obtained biologically active substances as potential agents having an antitumor effect on certain types of cancer cells, such as the following 6 tasks are displayed:

1. To increase the production of biologically active substances with potential antitumor action through the optimization of the conditions for growth and development of selected bacterial and algal strains.
2. To determine the possibilities of immobilization of the cells of the selected strains and to choose the most effective approach and a corresponding matrix.
3. To test the selected immobilization procedures for the cells of the bacterial and algal producers to increase the synthesis of the target biologically active substances.
4. To investigate the possibilities of the selected immobilized preparations for repeated application and evaluation of the obtained product.
5. To develop a comprehensive scheme for the preparation by including immobilization, isolation and testing of antitumor properties of the obtained glycolipid biosurfactants from bacteria and the exopolysaccharides obtained from algae in *in vitro* experiments.
6. To evaluate the effect of the obtained glycolipids and polysaccharides on the survival of healthy and some cancer cell lines.

## **Material and methods**

This part of the dissertation is distinguished by an extremely detailed and competent presentation of the structure of the study. The methodical approach used is modern and adequate

to fulfill the aim and the tasks set. For the needs of the present study, mag. pharm. Angeliki Konstantinidou has included glycolipids isolated by testing different genera of bacteria from the collection of IMikB, BAS - (Rhodococcus, Micrococcus, Nocardia, Pseudomonas, etc.). The selection of the isolates was carried out according to the presence of surface activity of the obtained substances. The Ph.D. candidate isolated the strain *Pseudomonas aeruginosa* BN10 from soil contaminated with hydrocarbons. *Pseudomonas aeruginosa* BN10 and the other selected producer strain *Rhodococcus wratislavensis* were propagated by a standard enrichment technique and selected for their ability to produce surface-active glycolipid-type compounds. Both strains were maintained on nutrient agar slants (Difco Laboratories Inc., Detroit, MI, USA) at 4 °C. Mag.-Pharm. Angeliki Konstantinidou incubated the cultures on a shaking shaker (120 rpm) at 30 °C for 7 days. The resulting culture broth was centrifuged at 8000 g for a period of 20 min.

The supernatant was acidified with 6M HCl and allowed to stand at 4 °C overnight. After a next centrifugation at 12,500 g for 20 min, the pellet was collected. The selection of isolates was carried out according to the presence of surface activity of the obtained substances.

Cultivation was carried out in a mineral salt medium containing (g/L): K<sub>2</sub>HPO<sub>4</sub> 3H<sub>2</sub>O (4.8); KH<sub>2</sub>PO<sub>4</sub> (1.5); (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (1.0); Na<sub>3</sub>(C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>)<sub>2</sub>H<sub>2</sub>O (0.5); MgSO<sub>4</sub> 7H<sub>2</sub>O (0.2); yeast extract (0.1) supplemented with a micronutrient solution with the following composition in (mg/L): CaCl<sub>2</sub> 2H<sub>2</sub>O (2.0); MnCl<sub>2</sub> 4H<sub>2</sub>O (0.4); NiCl<sub>2</sub> 6H<sub>2</sub>O (0.4); ZnSO<sub>4</sub> 7H<sub>2</sub>O (0.4); FeCl<sub>3</sub> 6H<sub>2</sub>O (0.2); Na<sub>2</sub>MoO<sub>4</sub> 2H<sub>2</sub>O (0.2) and 2% n-hexadecane.

## Results

The results of the trial conducted are well presented and highly illustrated with 42 figures. Moreover the results are presented, analyzed, and compared in absolute values. Mag. Pharm. Angeliki Konstantinidou has carried out a selection of bacterial producers of glycolipid biosurfactants - *Pseudomonas aeruginosa* and *Rhodococcus wratislavensis*.

The use of the immobilization method and the selection of a suitable matrix - the polyethylene oxide cryogel matrix in the selected bacterial strain *Pseudomonas aeruginosa* proved to be suitable, and the use led to an increased synthesis of rhamnolipids. The Ph.D. candidate proved a decrease in the survival of the tested two cancer cell lines, after applying different concentrations of the synthesized bacterial product, most pronounced on the highly metastatic cell line - MDA-MB 231, with an insignificant decrease in the effect on the normal cell line MDA-MB 231, with an insignificant reduction of the action on the normal cell line - MCF-10A.

Mono-rhamnolipids are more effective than di-rhamnolipids. The cryogel matrix synthesized on the basis of polyacrylamide leads to increased production, it turns out to be the most suitable for the immobilization of *Rhodococcus wratislavensis*. After immobilization in the HEC cryogel matrix resulted in an increase in polysaccharide production from both tested strains. Mag.Pharm. Angeliki Konstantinidou observed a significant decrease in the viability of cancer cell lines depending on the concentration of heteropolysaccharide from both red microalgae,

with a stronger effect found in the highly metastatic breast cancer cell line MCF-7 and almost no inhibitory on the normal cell line. In her trial the Ph.D candidate confirmed the significant antiproliferative and cytotoxic effect of Porphyridium cruentum polysaccharide, having also demonstrated changes in the nuclear morphology of MCF-7 tumor cells, including chromatin condensation, nuclear fragmentation and formation of apoptotic bodies.

### **Conclusions and scientific contributions**

The personal participation of mag. pharm. Angeliki Konstantinidou in the conducted trial is indisputable. The conclusions achieved by the Ph.D. candidate are 13 and have a logical sequence based on the competent analysis of the results obtained. The Ph.D. candidate has singled out a total of 7 original contributions, and they are of a confirmatory and scientific-applied nature.

### **Ph.D thesis abstract and scientometric indicators**

The Ph.D. thesis abstract is properly structured and contains 56 pages, 42 figures, adequately reflecting the essential elements of the dissertation and the results obtained. In connection with the dissertation work mag. pharm. Angeliki Konstantinidou has submitted a list of 2 publications, one published in a PubMed-indexed Journal registered in the second quartile (Q2) and the second article in the third quartile (Q3). The Ph.D. candidate fully covers the mandatory minimum according to this criterion, i.e., 30 points.

### **Critical notes and recommendations**

It's impressive that in the Ph.D. thesis of mag.-pharm. Angeliki Konstantinidou, the results are merged in a common chapter (V. RESULTS) with the discussion, without this being reflected in the content, and in the abstract, they appear united in chapter III. (RESULTS AND DISCUSSION). In order to comply with the academic standard for dissertations, the content of the Ph.D. abstract thesis follows the sequence and structure of the dissertation and vice versa, except for the literature review. Additionally, the Ph.D. candidate failed to distinguish the nature of her contributions in the following categories: essential scientific contributions, scientific-applied contributions, methodological contributions, and contributions of a confirmatory nature. The remarks made in no way diminish the qualities and significance of the present Ph.D. thesis.

Finally, I would recommend the mag.-pharm. Angeliki Constantinidou to issue a monograph dedicated to the production of biologically active substances from bacteria and algae and evaluation of their potential action as antitumor agents in vitro experiments. Such has not yet been issued in Bulgaria and would be extremely useful for biologists, microbiologists, biochemists, and other scientific workers.

## **Conclusion**

I strongly believe that the dissertation of mag. pharm. Angeliki Nikolaos Konstantinidou is up-to-date and properly structured with excellent methodology and credible results. It contains scientific and scientific-applied results that represent an original contribution to biochemistry and biotechnologies and fully meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, as well as the Regulations of the Institute of Experimental Morphology, pathology, and anthropology with a museum of the BAS. The presented materials and the dissertation results fully correspond to the specific requirements adopted in connection with the IEMPAM-BAS Rules for the application of the LDASRB.

The Ph.D. thesis illustrates that the mag. pharm. Angeliki Nikolaos Konstantinidou possesses broad and deep theoretical knowledge while demonstrating advanced competencies and potential for independent conduct, follow-up, and coordination of scientific research.

Due to the above-mentioned, I confidently give my positive assessment of the Ph.D. thesis, proposing to the honorable scientific committee to award the Pharm. Angeliki Nikolaos Konstantinidou, the educational and scientific degree "doctor" in professional direction 4.3. Natural sciences, scientific specialty Biochemistry.

**30.08.2022 г.**

**Reviewer:**

A red rectangular box redacting the signature of the reviewer. There are blue ink scribbles above and below the box.

**Sofia**

**Prof. Dr. Ioannis Vassilios Papathanasiou, MD, Ph.D.**