S T A N O V I S H E

by Assoc. Dr. Adriana Georgieva Guscherova
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Regarding: dissertation work of Milena Nikolova Petrova for awarding the educational and scientific degree "Doctor" in professional direction 4.3. Biological sciences, Scientific specialty "Microbiology" on the topic: "Development of biological products from new natural sources"

Scientific supervisors: Prof. Dr. Petya Hristova and Assoc. Dr. Ganka Chaneva appointed by an order for the defense of a dissertation presented by Milena Nikolova Petrova on 07/05/2022 with protocol No. RD 38-389/07/13/2022 .date by the chairman of the jury, agreed with the dean of the faculty

Doctoral student Milena Nikolova Petrova submitted all necessary materials, references and documents for the defense procedure according to the requirements of the Faculty of Biology, SU "St. Kliment Ohridski".

The dissertation is written on 160 pages. Its layout is very well balanced in terms of the volume of the individual sections: introduction, literature review, aim and objectives, materials and methods, results, conclusions and contributions. They are filled with specific content, respecting the ratio of individual parts in such a scientific work.

The results are presented logically and clearly in 41 tables and 26 figures. The appendices are 26 pages containing 12 tables and 28 figures. The bibliographic reference includes 477 sou The abstract is written on 55 pages.

It impresses with its firmness and purposefulness. It reflects a sufficient number of citations from publications that are directly relevant to the tasks set in the dissertation work.

In recent years, there has been a significant increase in interest in the study of biological effects of natural compounds against bacterial or fungal infections.

The purpose of the dissertation work is well formulated and the tasks to be performed are clearly outlined: research and evaluation of the antioxidant and antimicrobial activity of bioactive substances from new natural sources - products from microalgae and invertebrates (hemocyanin). Identification and cultivation of promising strains of microalgae was carried out, their antioxidant and antimicrobial activity of natural antibiotics in relation to phytopathogens, the antimicrobial activity of hemocyanin in relation to human pathogens was determined.

Three different types of microalgae were used in the dissertation - the cyanobacteria Arhtronema africanum Lukavský 1981/01, Nostoc commune Vaucher and an isolate from a chlorella-like microalgae (HPV-A1).

To determine the antimicrobial activity in the dissertation, test microorganisms from the collection of the "General and Industrial Microbiology" department were used.rces. Optimization of the conditions for growth and development of the selected algal strains was achieved.

Cultivation of the strains was accomplished by standard methods. Regarding the identification, genetic-molecular studies were carried out. PCR amplification was performed with universal and specific primers.

The biochemical composition of the microalgae was studied; the content of proteins, carbohydrates, lipids and pigments, as well as the antioxidant activity of microalgae extracts.

The antimicrobial activity of microalgae extracts was determined by the Bauer-Kirby method, and detailed information was provided for various microalgae extracts. A new method

was used to determine the zone of inhibition by photographic material against a wide range of phytopathogenic bacteria and fungi.

The newly isolated HPV-1 strain was identified phenotypically as a representative of the family Chlorellaceae and after sequencing of amplified regions of 18S DNA, showing close homology to the genus Muriella.

The obtained data on the antimicrobial activity of Arthronema africanum and a newly isolated strain of green microalgae HPV-A1 against human pathogens and phytopathogens are made for the first time.

• New information was obtained on the antioxidant activity of microalgae extracts; From the review of the experimental techniques, it can be concluded that in the process of her studies the doctoral student acquired the qualities of a qualified researcher. A wide range of classical and modern molecular biological methods have been mastered and applied. It is obvious that the PhD student has sufficient molecular biological knowledge and the methods used are described and explained in detail. The applied methods correspond to the set goals and tasks. The discussion is developed adequately to the obtained results and is in accordance with the literary data from the used bibliography.

9 conclusions and 5 contributions were derived and precisely formulated, which reflect the results and fully meet the tasks set. I agree with the author's assessment of the developed contribution of the dissertation work.

In connection with the dissertation, the doctoral student has presented 2 publications, in journals with an impact factor, referenced in the Web of Science database.

In one of the articles, Milena Nikolova Petrova is the first author, in the other, the third author. There is also an article in a journal without an impact author (IF). She is involved in 2 research projects related to the dissertation and another 3 projects indirectly related to the dissertation labor. The presented scientific works fully cover the subject and contain the results of the conducted research. For the period of the development of the dissertation, the doctoral student completed the required set of courses during the doctoral studies.

The dissertation work "Development of biologically active products from new natural sources" is an in-depth study with scientific value and practical application.

When discussing the results, the high professional maturity and scientific competence of the doctoral student make an impression. The work establishes the author as a responsible and reliable researcher who can independently conduct research at a high scientific level and interpret complex scientific results. In conclusion, I would like to emphasize that it was extremely pleasant for me professionally to draft an opinion on the dissertation of Milena Nikolova Petrova

Conclusion:

The dissertation shows that the doctoral student Milena Nikolova Petrova is a well-rounded scientist who knows modern methods of interpreting the obtained results and fully meets the requirements for obtaining the educational and scientific degree "Doctor". This gives me reasons to recommend and confidently suggest to the esteemed jury to vote for awarding her "Doctor" in professional field 4.3 Biological Sciences, Scientific specialty "Microbiology"

Prepared the opinion:

Date Assoc. Dr. Adriana Guscherova