EXPERT OPINION

by Assoc. Prof. Denitsa Rumenova Teofanova, PhD,
SU "St. Kliment Ohridski", Faculty of Biology, Department of Biochemistry,
member of the Scientific Jury, appointed by Order No. RD-38-404 from 13.07.2022. of the Rector of SU "St. Kliment Ohridski", Prof. Anastas Gerdzhikov, for awarding the Educational and Scientific Degree "Doctor" in
Field of higher education: 4. Natural sciences, mathematics and informatics,
Professional field: 4.3. Biological Sciences
PhD program: Virology
REGARDING:
Dissertation topic: "Investigating the impact of physical and biological factors on the realization of herpes viruses"
Author of the dissertation: Venelin Ventsislavov Tsvetkov, full-time PhD student at the Laboratory of "Virology" of the Faculty of Biology, SU "St. Kliment Ohridski"
Scientific supervisor: Prof. Stoyan Angelov Shishkov, PhD

1. General presentation of the procedure and the PhD student

1.1.Documents

The materials submitted by Venelin Ventsislavov Tsvetkov include the following required documents according to the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Terms and Conditions for Acquiring Scientific Degrees and Holding Academic Positions at SU "St. Kliment Ohridski": 1) Dissertation; 2) Abstract in Bulgarian and English; 3) List of scientific publications related to the dissertation, which I think would be better if they were also provided in full text and an accompanying protocol for the contribution of co-authors; 4) CV; 5) Copy of the Master's degree qualification diploma and its annex; 6) Certificate of passed exams from the individual plan; 7) Plagiarism report, 8) Order for enrollment in the PhD program, lacking the order of extension; 9) Reports from the PhD student and his supervisor for admission to internal defense, lacking the order of dismissal with the right of defense; 10) Order for the appointment of a scientific jury and 11) Certificate of compliance with the minimum national requirements for ESD "Doctor".

The dissertation corresponds to the criteria for acquiring scientific degrees and holding academic positions in SU for professional field 4.3. Biological Sciences.

1.2.Introduction of the PhD student

Venelin Tsvetkov obtained a Bachelor's degree in Molecular biology in 2016 and Master's Degree in Virology in 2018 in the Faculty of Biology of the SU "St. Kliment Ohridski". In the period 2018-2022 is a full-time PhD student in the Laboratory of "Virology" of the Faculty of Biology. He gained additional professional work experience as a Biologist at the IMDL "Ramus" (2017-2019) and in the field of clinical trial monitoring from 2019 to date. All of the above show scientific interest combined with upgrading of knowledge, experience and skills. The attached CV could be supplemented with the PhD student's participation in scientific projects and forums, which, thanks to personal contacts with him, I am informed that he has.

1.3.Personal impressions of the PhD student

I have known Venelin Tsvetkov since 2018 as a result of collaborative professional contacts, which continue to this day, as colleagues from the Laboratory of Virology, where he is a PhD student, and the laboratory of the Department of Biochemistry, where I work. He has always shown curiosity, undoubtable desire to upgrade his knowledge and skills in different areas of science, hard work, dedication and persistence in every endeavor. His responsible attitude in the implementation of various activities related to his dissertation and in his participation in scientific research projects is indisputable. The serious progress that this colleague has made in recent years in terms of improving his qualification skills, evidenced by the completion of examinations during the PhD, including in a number of courses, the participation in scientific forums in the promotion of the achieved results and the training of undergraduate students within the framework of compulsory pedagogical activities, should be noted.

1.4.Plagiarism

Based on the plagiarism check annexes, it is clear that the detected similarity percentages for the three criteria do not affect the original results obtained and are not a consequence of illegal borrowing and copying of foreign text, but rather concern methodological descriptions that cannot be significantly modified.

2. Characteristics of the thesis

2.1. Relevance and significance of the dissertation topic

The relevance of the topic of submitted dissertation is indisputable and is determined by a number of factors. On the one hand, herpes infections are one of the most widespread worldwide and can cause diseases of varying severity and clinical presentation - from asymptomatic infections to severe generalized ones, meningitis, encephalitis and malignant formations. On the other hand, there are ways to fight these infections. A number of drugs used in this direction face many problems - viral strains that are not affected by them, the emergence of resistant strains, as well as side, unwanted effects on the body. A large part of the dissertation deals with an extremely modern, contemporary alternative to the above-mentioned drugs - phytoproducts. Plant biologically active substances are used in medicine, including folk medicine, for the treatment of various diseases, but as antiherpetic agents, they would be novel, with the potential for high efficiency and, not least, with better biological tolerance. In third place is the need, widespread in recent years in pandemic conditions, for adequate personal protective equipment to reduce the transmission of viral infections. Testing them and determining their degree of protection is also an integral part of current research. And last, but not least, there is the extremely innovative area of the application of high-tech methods based on the use of plasma as a method of treating viral infections. These technologies have enormous advantages, being non-destructive to tissues, safe and effective against bacterial and viral pathogens.

2.2. Structure of the dissertation, persuasiveness of the obtained results, interpretations, conclusions, and scientific contributions

The dissertation is prepared according to the generally accepted scheme. It is written on 123 standard printed pages and comprises the following sections, including sub-sections: Title

Page (1 page), Table of Contents (3 pages), List of Abbreviations Used (3 pages), Introduction (2 pages), Literature Review (42 pages), Aim and objectives (2 pages), Materials and methods (19 pages), Results and discussion (31 pages), Conclusion (2 pages), Final Conclusions (4 pages), Statement of contributions (1 p.), List of scientific publications related to the dissertation (1 p.), List of references (12 p.). The information presented in it is illustrated with 30 figures (18 of which in the "Results and Discussion" section) and 23 tables (16 of which in the "Results and Discussion" section), which are of sufficiently good quality.

The dissertation is written in appropriate scientific language, with a small number of foreign words, punctuation, technical and stylistic errors, and is of sufficient volume to acquire the PhD degree. There is an imbalance in terms of the optimal ratio between the sections, and it is recommended that the "Results and Discussion" section should dominate the "Literature Review", which is not the case here. A more positive impression would have been made by separating the "Results" and "Discussion" sections, which would show the PhD student's ability to interpret the obtained data and distinguish the obtained results from their discussion, which is an important skill acquired at this level of education. The literature used includes 155 sources (4 in Cyrillic, 149 in Latin and 2 links from Internet sources) and the way the bibliographic reference is presented is not uniform for all of them. The use of sites such as Wikipedia as an adequate source of information is not recommended. In general, a certain discrepancy between the cited source used and the information appearing in the text, as well as repetitions of information in different sections, is noticeable in the work. Necessary citations are missing in places, and not all sources listed in the bibliography are cited in the text.

The introduction is well structured, demonstrates the importance of the subject matter, provides information about the object of study and the innovativeness of some of the approaches, and outlines the foundations on which the work rests.

The literature review is extremely detailed and covers all aspects of the subject matter on which the work rests. It includes detailed data on herpesviruses, starting from historical background, current information on classification, morphology, genomic organization, replicative cycle of HSV and the main characteristics of representatives of individual subfamilies, as well as the pathogenesis and immune response against these viruses and their strategies to avoid the body's defense mechanisms. Options for the therapy and prevention of herpes infections are also covered, including phytotherapy and plasma application options in medicine, showing their modes of action, advantages, and disadvantages. It becomes clear that the PhD student is well acquainted in the subject matter and in addition to good theoretical background, his ability to work with scientific literature to systematize the available information is proven.

The aim of the dissertation is clearly formulated and hindered by the relevance of the topic. ***The tasks*** related to the planned scientific research activities to achieve the stated aim are also adequately set.

In the "***Materials and methods***" section, the methods used are described in detail, it is evident that they are adequately selected and meet the set tasks. They are presented in a way suitable for reproducing the experiments. However, some of them lack corresponding citations. The information on the plant extracts used is not sufficiently clear and complete (this applies in particular to Table 7) and is not uniform for the three plant species. Regarding the protection

capacity of personal protective equipment, data on specific ones are only available in the "Results and Discussion" section, and I think it is more appropriate to include them in this section as well. For greater clarity, a brief description of the principle purpose of the techniques used could be given. Despite my remarks, the large range and variety of learned techniques is clearly evident, which indicates a good methodological background of the PhD student.

The results obtained from the conducted research are described consistently and in accordance with the tasks set in the ***"Results and Discussion"*** section. The influence of extracts from *Vaccinium vitis-idaea L.*, *Astragalus glycyphyllos L.* and *Artemisia chamaemelifolia Vill*. on both replication and extracellular virions of human alphaherpesvirus type 1 strain F and acyclovir-resistant human alphaherpesvirus type 2 strain DD is shown. The strongest protective effect of *Vaccinium vitis-idaea L*. from the Balkan Mountains on HSV 2 (DD) was reported. No effect of the extracts on extracellular virions was observed. The effectiveness of manufactured and marketed filtering face masks in terms of their protective potential to reduce the transmission of viral infections is defined. It is shown that FFP-type filtering face masks provide the highest protection compared to public face masks. The effect of plasma-activated culture medium and distilled water on the replication and extracellular virions of HSV-1 strain F is also given. It was found that the treated medium did not protect the virus-inoculated cells, but the virucidal effect of plasma-treated viral suspension diluted 1:2 with dH2O was observed by a decrease in the titer of the viral sample. A rather laconic interpretation of the data obtained is presented, especially regarding the effect of the plant extracts. Preferably, the relationship between the results and explanations for them should be made more explicit. A more convincing discussion is needed, at least based on the data available in the literature of recent years. Based on the obtained results, 19 ***conclusions*** and three ***contributions*** were formulated. The conclusions follow logically from the results of the conducted studies, but could be more concisely, clearly and definitively formulated, while some overlap with the results is observed in them. I fully accept the contributions presented in the dissertation.

2.3. Correspondence between the Abstract and the dissertation

The abstract is prepared in accordance with the requirements and presents in an abridged version the study, the experimental work, the results obtained and the conclusions and contributions made on their basis. The only discrepancy is observed in the number of presented conclusions - 19 in the dissertation and 16 in the abstract, while they should be identical.

3. Questions for the PhD student

I have the following questions for the PhD student:

1. Why did you choose to study the effect of plasma-source activated medium and distilled water only on the replication and extracellular virions of HSV-1 strain F but not HSV-2 (DD) as for the plant extracts?

2. Does it make sense to study the combined antiviral effectiveness of the plant extracts and what is the probability of a negative effect on the cells and/or a lack of protective action due to the influence of the relevant solvents (DMSO, methanol, chloroform)?

3. Is it possible to use the plasma source in a direct way on a cell monolayer?

4. Based on what has been achieved so far and considering the still unsolved problems regarding the fight against herpes infections, in what direction do you think future research should be directed?

4. Conclusion

In conclusion, I believe that the presented dissertation represents an in-depth study on an undeniably relevant topic, incorporating modern and innovative techniques and bringing with it corresponding contributions to the scientific field. It demonstrates the PhD student's sound theoretical background and the acquired wide range of methodological skills in the analysis and solving of problems. Regardless of the remarks made, I believe that the dissertation fully complies with the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as with the Regulations for the Terms and Conditions for Acquiring Scientific Degrees and Holding Academic Positions at SU "St.Kliment Ohridski", for awarding the educational and scientific degree "Doctor". All of the above gives me grounds to positively evaluate the dissertation work of Venelin Tsvetkov and to recommend to the other members of the esteemed Scientific Jury to support the awarding of the educational and scientific degree "Doctor" in the professional field: 4.3 Biological Sciences (Virology) to Venelin Ventsislavov Tsvetkov.

05. 10. 2022 Prepared the expert opinion:
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Sofia /Assoc. Prof. Denitsa Teofanova, PhD/