Attention of the members of the scientific jury,

determined by Order No. RD-38-543/18.08.2023,

issued by the Rector of SU"St. Kliment Ohridski" - Sofia

R E V I E W

of the scientific production of chief assistant Dr. Kalina August Shishkova, PhD, "Virology" Laboratory, Faculty of Biology, SU "St. Kliment Ohridski", Sofia

in connection with the procedure for occupying the academic position of "ASSOCIATE PROFESSOR" in direction 4.3 Biological sciences (Virology - molecular virology), announced in State Gazette No. 65 of 28.07.2023

written by Prof. Dr. Radka Mladenova Argirova, medical doctor-virologist, speciality "virology", Acibadem City Clinic UMBAL "Tokuda", Sofia

DEAR RESPECTED MEMBERS OF THE SCIENTIFIC JURY,

Here I present to your attention a review in connection with the announcement in the Official Gazette, No. 65 of 28.07.2023, of competition for the academic position of "associate professor" at the "Virology" Laboratory, BF, at the University of St. Kliment Ohridski" - Sofia and according to Order No. RD-38-543/18.09.2023, issued by the Rector of SU "St. Kliment Ohridski" - Sofia.

Chief Assistant Dr. Kalina August Shishkova was born in 1970 in Sofia. In 1997 she graduated as a Magister at Sofia University "St. Kliment Ohridski", Faculty of Biology, specialization "Molecular Biology". In 2014, she defended her doctor thesis titled : "Distribution and genetic diversity of Torque teno viruses (family Anelloviridae) in Bulgaria". Until now, her work is the basic one in Bulgaria, concerning this relatively new and unknown group of hepatitis viruses - family Anelloviridae. From 1997 to 2002, she was a specialist-virologist at the Stefan Angelov Institute of Microbiology, BAS, after which she held the following positions: part-time assistant, assistant, senior assistant and chief assistant at the Virology Laboratory, Faculty of Biology, SU "St. Kliment Ohridski", Sofia. She is excellent in English language.

Chief assistant Dr. Kalina August Shishkova is mainly engaged in teaching and research activities, often the latter transforms into an expert activity. In the current competition, **Dr. Shishkova presents 1 dissertation (50 points according to the quartile system), 33 scientific articles (358 points according to the quartile system), 138 citations, (60 points according to the quartile system; the minimum 30 citations according to the national requirements), participation in 20 scientific projects, supervision of 9 diploma theses of graduating magisters Program in Virology, of which she has been secretary since 2009.**

According to the candidate herself, her scientific works contain contributions in the field of experimental and medical virology and can be divided thematically into the following four directions: I. Screening of natural products for antiviral activity, II. Research of newly synthesyzed compounds for antiviral activity, III. Medical virology, IV. Study of the antiherpetic effect of physical factors.

One of the main and long-standing directions in the activity of the laboratory of virology at the Faculty of Biology is the search for new antiviral, in particular antiherpes substances of different natural origin - plant, animal, microbial. This is imposed as a constant task and requirement in modern medicine due to the increasingly frequently observed resistance and allergic reactions when using standard antiherpetic agents. A large number of extracts, as well as their fractions and active substances isolated from lower and higher plants, invertebrates and microorganisms, have been studied for the presence of inhibitory and inactivating activity. Their antiviral effect was determined against Herpes simplex virus type 1 /HSV-1/, Herpes simplex virus type 2 /HSV-2/ and acyclovir resistant strain. A strong antiherpetic effect of the methanolic and aqueous extracts of laboratory-grown plants from the *Lamiaceae* family has been demonstrated /publications 1,2/. An anti-herpes effect has also been proven for standardized extracts from a number of Bulgarian plants and herbs, many of which are used in folk medicine - publications numbered 4,5,6,7,810,11. The published review on the

phytochemical composition of cultivated plants and their antiviral effect /3/, cited 20 times, is particularly valuable. For the first time, the antiherpetic action of hemocyanins (as whole molecules or as structural and functional subunits) isolated from the Black Sea rapana (*Rapana hemocyanin*) and representatives of the genus *Mollusca, Eriphia verrucosa (hEv), Helix aspersa (Ha)* and structural subunit α was demonstrated for the first time -HaH from H. *aspersa* hemocyanin (sHa) to HSV-1 replication /12,13/. The antiherpetic activity of secondary metabolites isolated from **Lactic Acid Bacteria** isolated from fermented products /14/ has been investigated and proven, some of which are promising for future studies due to their high selective index.

The rational synthesis of new substances with anti-herpes, anti-influenza and anti-coronavirus action is based on the knowledge of the structure and functions of the applied antivirals. For example, the molecular structures of the newly synthesized analog compounds of the ion channel inhibitors amantadine and rimantadine were thoroughly investigated using single crystal X-ray analysis. Molecular docking studies showed that two of the investigated compounds, namely 2A and 4A, showed promising binding affinity to HCoV-229E and 2 SARS-CoV-2 sites - RNA-dependent RNA polymerase site and SARS-CoV-2 Nsp3 (207-379, MES site) respectively /21/. This article was published in 2023, it's still early for citations, but I'm sure it will resonate widely in the scientific community.

The most important contribution in the field of "Medical virology" is the demonstration for the first time in the country of Torque Teno viruses by adapting different primer systems. The presence of the viruses was proven in samples from blood donors, patients with registered viral hepatitis, patients with a primary brain tumors, patients with respiratory diseases, kidney transplant patients and patients of unknown disease etiology. I have already said that this is still one of the few studies in our country.

In this direction, the establishment of human papillomavirus (HPV) in a risk group of patients positive for the presence of periodontitis-causing bacteria is also important. The most frequently detected genotype of the papillomavirus associated with periodontitis is HPV58.

All studies are performed with reliable and modern virological and molecular biological research methods, they are up-to-date, with the prospect of being continued in the future, they were carried out in a team of different specialists, as evidenced by the participation in 20 projects with departmental and external funding, which fully corresponds to the interdisciplinary nature of modern science, the conclusions are convincing.

The teaching and pedagogical activities of Kalina Shishkova include lectures since 2002 on Virology - OKS "Bachelor"; "Antiviral Immunity", "Phages and Lysogeny" - OCS "Master", practical exercises and educational practices in "Molecular Virology", "Virology" and "Microbiology and Virology". Kalina Shishkova is also a co-author of the "Guide for practical exercises in virology", which is omitted from the list of publications , but important for the process of training and work in the laboratory of virology.

I fully agree with the distribution into 4 groups of the main topics of the candidate's scientific activities, as well as with the conclusions and contributions of her research. Since in her development Kalina Shishkova has inherited and continues to develop the directions of antiviral therapy with natural substances, it is quite understandable , that she has expanded this field of research with an active search for analogues of known antivirals in order to improve their antiviral activity and reduce resistance and adverse events in the therapy process. **Therefore, there is continuity in thematic respect, combined with updating the research methods, expanding the scope of the target natural sources - the object of the research and enriching the previously known conclusions with new contributions from these researches**.

The academic position "associate professor" requires serious teaching work, which is the first duty in a university, in this case - BF. Kalina Shishkova's teaching and workload includes both teaching students, with exercises and lectures, as well as guiding masters in developing their diploma theses. Kalina Shishkova is a supervisor of 9 graduates.

I personally know Dr. Kalina Shishkova as a thorough, accurate researcher with lasting interests in virology, which is also proven by her scientific output.

In conclusion, the comprehensive evaluation of the candidate's research and teaching activities, contributions and participations and in promoting scientific achievements in her field, her long experience in teaching modern virology, as well as her personal qualities, give me the confidence to vote positively for the appointment of the main assistant Dr. Kalina August Shishkova, Ph.D., to the academic position of "associate professor" in the Faculty of Biology of the University of St. Kliment Ohridski", Sofia. Her achievements exceed the minimum required for an "associate professor" position, so I strongly propose that the members of the esteemed scientific jury also vote positively.

Sofia, Nov.6th, 2023 REVIEWER:

София, 05.11.2023 РЕЦЕНЗЕНТ: