

by Prof. Rayko Dimitrov Peshev, D.Sc, Dept. "Epizootology and Infectious Diseases of Animals" in National diagnostic and research veterinary medical institute, Sofia, according to the competition announced in the State Gazette, issue 11 of 07.02.2025 for the academic position of "Docent" in the field of higher education 4. Natural Sciences, Professional field 4.3. Biological Sciences (specialty Virology), announced for the needs of the Laboratory "Experimental Chemotherapy of Influenza", Department of Virology, Institute of Microbiology "Stefan Angelov" – Bulgarian Academy of Sciences.

In connection with the competition announced by the Institute of Microbiology "Stefan Angelov" of the Bulgarian Academy of Sciences, Sofia, for the needs of the Department of "Virusology", Laboratory "Experimental Chemotherapy of Influenza", for the academic position of "Docent", the documents have been submitted by the sole candidate, chief Assistant d-r Lora Simeonova Simeonova. The documents required for the competition are: abstract of a dissertation for the educational and scientific degree doctor, autobiography, declaration of originality, diploma for the educational and scientific degree doctor, review of scientific research activities and vision for future work, publications related to the acquisition of the educational and scientific degree doctor, abstracts of articles in Bulgarian and English, certificate of fulfillment of the minimum national requirements according to the Law of development of academic staff in republic of Bulgaria, certificate of fulfillment of the additional requirements of the Institute of Microbiology of the Bulgarian Academy of Sciences, certificate of teaching activity, certificate of contributions, reference of written reviews, reference of participation in scientific research projects, reference of participation in scientific forums, certificate of work experience, application for participation in a competition for Docent, advertisement in the state newspaper.

Scientific and publication activity

The dissertation for the degree of Doctor of Sciences of chief Assistant d-r Lora Simeonova was defended in 2011 and is on the topic: Study of the combined effect of rimantadine and oseltamivir against influenza A virus. The abstract presents 2 titles of scientific publications and 9 scientific reports presented at Bulgarian and world congresses and conferences, which have already been reviewed and used in the acquisition of the general educational and scientific degree "doctor".

In connection with the announced competition for Docent, senior assistant Lora Simeonova presents 20 publications that have been published in journals with an impact factor and with reviewers. The most important results and contributions can be divided into the following areas:

The anti-influenza drugs rimantadine and oseltamivir in suboptimal and optimal doses can be combined effectively to prevent the severity of influenza infection, reducing the mortality and lung pathology of mice experimentally infected with influenza A/H3N2 virus during a five-day prophylactic and therapeutic course of administration of both substances (scientific article 1).

Combining the antiviral drug oseltamivir with α -Tocopherol acetate affects the antioxidant defense in the host and reduces the severity and mortality of influenza virus infection. This strategy can also be applied to other respiratory infections in which there is over activation of the immune system and active generation of toxic radicals (article 2).

Scientific article 3 compared the in vitro antioxidant capacity of several synthetic and natural compounds used and studied for the treatment of influenza – oseltamivir, isoprinosine, ellagic acid, vitamin E, and vitamin C.

Scientific article 4 is a review of current information on the most common viral pathogens causing serious economic losses in fish farming, with an emphasis on the main fish species farmed in the country - carp, catfish, trout, sturgeon (4).

In scientific paper 5, data are provided on the therapeutic effect on the survival of infected mice after administration of a viral inhibitor, administered together with an immunomodulator and an antioxidant. It has been shown that the effect was most pronounced with the triple combination – Oseltamivir, Isoprinosine, Ellagic acid (5).

Scientific communication 6 provides data from the application of classical and molecular techniques to assess the sensitivity of influenza virus strains (H1N1) and (H3N2) isolated in Bulgaria from 2004 to 2007 to neuraminidase inhibitors and M2 blockers. RT-PCR and sequencing were performed, through which gene segments HA, NA and M2 were analyzed, and it was found that out of 26 influenza strains (H1N1) and (H3N2), 22 were sensitive and 4 (two H1N1 and two H3N2) were resistant to rimantadine hydrochloride in the setting of CPE inhibition. 17 isolates were subjected to fluorescence analysis with IC₅₀ of zanamivir ranging from 1.05 nM to 5.28 nM, and for oseltamivir from 0.28 nM to 1.31 nM. After sequencing, mutations in the transmembrane region of the M2 protein responsible for resistance to adamantanes in the A/Sofia /1250 (H3N2) strain were identified. The virus has retained sensitivity to neuraminidase inhibitors. No mutations associated with resistance to M2 blockers or neuraminidase inhibitors have been found in any other viruses (6).

Scientific communication 7 presents results from determining the antioxidant activity of a polyphenolic extract from *Geranium sanguineum* in chemiluminescent model systems and studies the antioxidant activity of the enzymes superoxide dismutase and glutathione reductase. It was found that the polyphenol complex reacts against all tested reactive oxygen species, has pronounced chelating properties towards Fe²⁺ and that the reference concentration (0.1 mg/ml) is most suitable for therapeutic purposes.

In article 8, the in vitro antiviral activity of an ethanol extract of aerial parts of *Tanacetum vulgare* L. against viral strains from three taxonomic groups: coxsackie virus B1 (family *Picornaviridae*), herpes simplex virus type 1 (family *Herpesviridae*) and influenza A virus (family *Orthomyxoviridae*) was studied. The crude extract of *Tanacetum vulgare* L. was found to exhibit low cytotoxicity in Hep-2 and MDBK cells and moderate cytotoxicity in MDCK cells. The ethanol extract has significant antiviral activity against HSV-1, inhibiting viral replication, blocking viral entry into the absorption stage, and direct virucidal effects on extracellular virions. The observed effect of *Tanacetum extract* in vitro on influenza A H3N2 virus infection was weaker. It exhibited virucidal and adsorption-inhibitory activity, but lacked inhibitory effects on viral replication against CBV-1 (8).

In a scientific paper 9, the in vitro antiviral activity of 11 postbiotic samples (lysates or cell-free supernatants – CFS) obtained during the fermentation of six probiotic strains of *Lactobacillus* isolated from Bulgarian fermented milk products against Herpes simplex virus type 1 (HSV-1) and selected *Lactobacillus* strains were found to demonstrate strain-specific effects against HSV-1. These postbiotics affect different stages of viral infection in cell cultures.

In scientific communication 10, a "green" synthesis of lactose octaacetate by microwave irradiation was performed and its biological activity was studied. It was found that this compound showed activity with SI = 2.4 only against PV-1, but against HSV-1, that this compound showed activity with SI = 2.4 only against PV-1, but was inactive against HSV-1, IAV/H3N2 and Coxsackievirus B1. The authors believe that lactose acetates can be applied as

antimicrobial and antiviral substances in food, pharmaceutical, agricultural and cosmetic preparations (10).

Scientific paper 11 provides data on the chemical composition, antioxidant activity, antibacterial, antiviral, and acetylcholinesterase inhibitory properties of *Carlina acanthifolia* essential oil. *Carlina acanthifolia* essential oil demonstrated antiviral activity against human poliovirus-1 (LSc-2ab) by inhibiting the adsorption of viral particles onto cell lines (human epithelial type 2). The pharmacological activity demonstrated by *C. acanthifolia* radix essential oil indicates its potential application as a therapeutic agent.

In a scientific article 12, nine postmetabolites from lactic acid bacteria (LAB) of human or dairy origin were investigated for their antiviral activity against KOI herpesvirus using the cytopathic effect inhibition assay. Compounds produced by lactobacilli during fermentation, cultured on different media and harvested at different times, significantly inhibited extracellular KHV virions.

In scientific article 13, the authors set out to evaluate the in vitro anticarcinogenic, antiviral, and antioxidant effects of a crude methanol extract of the leaves of *Rhus typhina* L. The anticarcinogenic capacity of the extract was evaluated on two human breast cancer derived cell lines (MCF7 and MDA-MB-16 231) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay, and its effect on inhibiting cancer cell growth was compared with its action on a non-chemical breast epithelial cell line (MCF10A). The antiviral in vitro activity of the plant extract was also studied against viral strains from three taxonomic groups: herpes simplex virus type 1 (HSV-1), coxsackie virus B1 (CVB1) and influenza A virus (IAV/H3N2), using cytopathic effect inhibition tests, evaluation of the cytotoxicity of the extract by the neutral red uptake method, virucidal test, virus attachment test and pretreatment of cells with the extract. The plant extract has antiproliferative activity against the tested cell lines, with the effect being stronger on cancer cells. A moderate inhibitory effect on IAV/H3N2 replication was found, as well as an effect on herpes viruses and on the adsorption of HSV-1 and IAV/H3N2 strains, as well as a protective effect on uninfected cells before contact with HSV-1. The leaf extract of *R. typhina* also showed strong free radical scavenging activity.

In scientific communication 14, the potential synergism of chlorhexidine-silver nanoparticle conjugates against influenza virus type A, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* was investigated. Silver nanoparticles (SN) were obtained by reduction of silver ions with total phenolic extract of green tea and conjugated with chlorhexidine (Cx). A stable, negatively charged silver nanocolloid was obtained. The conjugates (SN-Cx) demonstrated potentiation of their effects against *S. aureus* and *C. albicans*, as well as synergism against *E. coli*, with minimum inhibitory concentrations of SN of 5.5 µg/mL + Cx of 8.8 µg/mL. SN exhibit excellent virucidal properties that are enhanced and show low toxicity. The binding of chlorhexidine to silver nanoparticles did not reduce its cytotoxicity on different cell lines (MDCK, BJ and A549). The newly synthesized antimicrobial agent has a promising therapeutic spectrum.

Article 15 examines the potential of probiotics to reduce or even prevent Koi herpesvirus infection. It provides an analysis of the disease caused by Koi herpesvirus, its prevalence, and some of the conventional treatment methods. The potential of probiotics to improve growth rate and immune resistance in carp has been examined, related to the mucosal intestinal immune system as the first immune barrier in fish (15).

Scientific paper 16 presents studies of postmetabolites produced by lactobacilli as broad-spectrum inhibitors of herpes viruses in vitro. Cytotoxicity, inhibition of cytopathic effect, virucidal effect, influence on virus adsorption to cells, as well as the protective effect

of postmetabolites on healthy cells were evaluated. The inhibitory effect was more pronounced against HSV-1 than against KHV at all stages of the viral cycle studied.

In scientific communication 17, the anti-coronavirus activity of chitosan-stabilized liposomal nanocarriers loaded with natural extracts from the Bulgarian flora was studied. Plant extracts from *Sambucus nigra*, *Potentilla reptans*, *Allium sativum*, *Aesculus hippocastanum* and *Glycyrrhiza glabra* L were used. It has been established that nanoparticles do not directly affect the viral surface or cell membrane, but serve only as carriers of the active substances, with the established protection being due solely to the intracellular action of the extracts.

In communication 18 the combination of Poloxamer 407 (P407) and hydroxypropylmethylcellulose (HPMC) hydrosols is proposed as an in situ thermogelling vehicle for nasal delivery of chlorhexidine-silver nanoparticle conjugates (SN-CX). Tests were conducted for dispersibility, washout time, in vitro drug release, ex vivo permeation, and antimicrobial activity. Anti-infective activity was found against seasonal influenza virus and beta-coronavirus, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and other pathogens.

Scientific article 19 reports the results of studies on the presence of viral DNA in three patients diagnosed with benign warts in the oral cavity. For this purpose, a polymerase chain reaction (PCR) kit was used for qualitative detection of human papillomaviruses, with the target of the polymerase chain reaction being the viral genes E6, E2, E1 and L1. Positive and negative controls were used to validate the results, and the results showed the presence of viral DNA (HPV 6) in all tested samples.

Scientific article 20 provides an update on recent findings on epigenetically sensitive mechanisms affected in viral infections by highly pathogenic strains of influenza virus (IAV subtype H1N1 or swine flu and IAV subtype H5N1 or avian flu) and coronavirus (CoV) (severe acute respiratory syndrome (SARS)-CoV, Middle East respiratory syndrome (MERS)-CoV and SARS-CoV-2). Host-pathogen interactions can determine the clinical outcome of infection. In critically ill patients, viral load persists along with an abnormal immune response and severe pulmonary disease. Viruses can use the host's cellular mechanisms not only to reproduce, but also to alter the host's gene expression, creating an environment favorable to the virus.

Fulfillment of the minimum national requirements

According to the reference for the minimum national requirements, the candidate has 50 points for indicator A - 1, no points for indicator B, and 102 points for indicator C, with 100 points required, as well as scientific publications in journals, which are referred and indexed in WoS/Scopus are 2 in Q 1, 2 in Q 2 and 1 in Q4. Indicator D - sum of indicators from 5 to 10 are needed 220 points and she has 236 points, with 3 being Q1, 4 being Q2, 3 being Q3 and 3 being Q4. Under indicator D citations in scientific publications referenced and indexed in WoS/Scopus 60 points are needed, she presents 50 citations, which carry 100 points. It can be seen that the candidate meets the national criteria and the increased criteria of the Institute of Sciences of the Bulgarian Academy of Sciences.

Fulfillment of the additional requirements of the Institute of Microbiology

According to the reference for the additional criteria of the Institute of Microbiology, 20 publications (after "doctor") in journals with IF/SJR, monographs, chapters of monographs, proceedings of international forums, published in full text, in 5 of them she is the first or corresponding author. For the entire scientific career, there are 149 citations with 100 points required. The IF for the entire scientific career should be 20, and it is 48.03. According to the criteria of the IM, the H factor should be 5, and she has an H factor of 7. Participation in scientific projects with 3 projects required, she has participated in 9 projects -

8 national and 1 international. The report on the trainings conducted shows that in 2016, Senior Assistant Simeonova conducted 40 hours of exercises in the Master's program in Virology, "Pharmacy" program at Sofia University (SU) "St. Kliment Ohridski. In the same year, 2024, she also delivered 20 hours of lectures in English in the Master's program in Microbiology and Virology, specialty "Pharmacy" at the Faculty of Biology of Sofia University. In 2025, she delivered 20 hours of lectures in English in the Master's program in Microbiology and Virology, specialty "Pharmacy" at the Faculty of Biology of Sofia University. Chief assistant Simeonova has also written 29 reviews of scientific articles in prestigious journals. She has participated in 28 national and international scientific forums, 12 of which were with poster presentations and 16 with oral presentations.

Conclusion

The scientific and research achievements presented to me by chief assistant Lora Simeonova Simeonova, and the results obtained in the field of virology give me reason to conclude that she exceeds the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation of the Institute of Microbiology of the Bulgarian Academy of Sciences. The scientific indicators have been exceeded and meet the requirements for acquiring the academic position of "Docent". My personal impressions of chief assistant Simeonova as a researcher are excellent and this allows me to recommend to the members of the Scientific Jury and the members of the Scientific Council of the Institute of Microbiology of the Bulgarian Academy of Sciences, Sofia, to vote positively for her to obtain the academic position of "Docent" to chief assistant Lora Simeonova Simeonova - in the professional field 4.3 Biological Sciences (specialty Virology), announced for the needs of the Laboratory "Experimental Chemotherapy of Influenza", Department of Virology, Institute of Microbiology "Stefan Angelov" - Bulgarian Academy of Sciences.

10.06.2025
Sofia

Written by:
(Prof. Rayko Peshev, D.Sc)

На основание
чл. 2 от ЗЗЛД