R E V I E W

on the materials for participation in a competition for the academic position of "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Microbiology", for the needs of the Department of Infectious Microbiology, Laboratory "Bacterial Virulence, Resistance and New Antimicrobial Agents" of the Institute of Microbiology "Stefan Angelov" at the Bulgarian Academy of Sciences (IMikB-BAS), announced in the State Gazette No. 66/12.08.2025.

<u>**Candidate for participation in the competition:**</u> **Chief Assit. Prof. Dr. Lyudmila Lyudmilova Dimitrova,** Department of Infectious Microbiology, Laboratory of Bacterial Virulence, Resistance and New Antimicrobial Agents of the Institute of Microbiology of the Bulgarian Academy of Sciences

<u>**Reviewer:**</u> **Prof. D. Sc. Teodora Petrova Popova,** University of Forestry, Sofia, field of higher education "Agrarian Sciences and Veterinary Medicine", professional field: 6.4. Veterinary Medicine, scientific specialty "Episootology, Infectious Diseases and Prevention of Contagious Diseases in Animals", appointed as a member of the Scientific Jury by Order No. I-142/01.10.2025 of the Director of the Institute of Microology-BAS.

1. Brief biographical information about the candidate.

Lyudmila Lyudmilova Dimitrova graduated from the Faculty of Biology of Sofia University “St. Kliment Ohridski” in 2013 with the degree “Master” in Ecology and Environmental Protection. She has been part of the research team of the “Bacterial Virulence, Resistance and New Antimicrobial Agents” laboratory since 2013, when she began her scientific career as a doctoral student of Acad. Hristo Naidenski, DVMSc., Head of the Department of Infectious Microbiology (DIM) at the IMikB-BAS and of Acad. Vasya Bankova, DChSc., Laboratory “Chemistry of Natural Substances”, Institute of Organic Chemistry with the Center for Phytochemistry (IOCCPH) – BAS. During her dissertation work, she gained experience in the application of various phytochemical methods for the extraction of biologically active substances from plants, as well as in testing their antimicrobial activity and studying their mechanism of action with microbiological and molecular biological approaches. Then her interest arose in studying the antimicrobial activity of various extracts and compounds of natural origin, including assessing their quorum quenching potential (inhibition of the bacterial quorum sensing system) and testing their influence in animal models. Another scientific interest related to the successfully completed Master's program "Ecology" is the establishment of microbial diversity in various ecological niches - water, soil and animals, as well as their potential for application in biotechnology, such as isolating cellulose-degrading microorganisms and optimizing environmental conditions. From 2016 to 2020, L. Dimitrova held the academic position (AP) "Assistant" at the Department of Microbiology of the IMikB-BAS, where she gained research experience by participating in the implementation of research projects and responsibilities related to their conduct and reporting. In 2019, she defended her dissertation at IMikB-BAS and obtained the educational and scientific degree (ESD) "Doctor" in microbiology. From 2020 to the present, she is a Chief Assistant in the same department of IMikB "Stefan Angelov" - BAS, where she participates in the implementation of research projects. She has completed specialized courses within the dissertation program: "Modern electron microscopy techniques in biomedical research" with Prof. Marin Alexandrov and "Cultivation of animal cells" with Prof. Elena Nikolova, as well as training seminars on "Completion of the next-generation scientists workshop presented by Illumina & ELTA 90 for library preparation, sequencing and bioinformatics". Her work experience in the specialty is 9 and a half years. She is fluent in written and spoken English, German and Russian. Has digital skills in working with

Microsoft Office™ (Word™, Excel™ and PowerPoint™), EndNote, PhotoShop and GraphPad Prizm.

2. Compliance of the submitted documents and materials of the candidate with those required according to the Regulations for RAS at the Institute of Microbiology - BAS.

Chief Assistant Professor Dr. Lyudmila Dimitrova has submitted all necessary documents and materials for participation in the competition on paper and electronically in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations for the Terms and Procedures for Acquiring Scientific Degrees and Holding Academic Positions at the Institute of Microbiology - BAS.

3. Evaluation of the candidate's scientific, applied scientific and publication activities.

3.1. Participation in scientific, applied science and educational projects.

CIhief Asst. Prof. Dr. Lyudmila Dimitrova has participated as a member of the working group in **26** research projects (25 - completed and 1 - ongoing), which is a **significant number.** She has led three of them. She was temporarily the head of one project at the Scientific Research Fund (KP-06-N36/7 of 13.12.2019) only during its first stage, due to her maternity leave in the period 2021-2023. Eleven of the projects are funded by the Scientific Research Fund, two - by the Bulgarian Academy of Sciences, another two - by the Ministry of Education, four - by universities, one is funded by inter-academic contracts and agreements, another - by a company, two - by European and international programs and funds and two - under other programs. These data show active research activity, which presents her as a **researcher with significant experience in the following contemporary areas:** • Determination of antibiotic sensitivity of microorganisms of bacterial origin to compounds of synthetic and natural origin, incl. by microtitration method and agar-diffusion methods; • Determination of cytotoxic and antineoplastic effect of natural biologically active substances and plant extracts *in vitro,* as well as acute and subacute toxicity *in vivo*, incl. and determination of skin irritation and sensitization; • Preparation and observation of microscopic preparations for scanning, transmission and fluorescence microscopy; • Methods for studying quorum sensing in bacterial communities as a way to study the antibacterial mechanism of action of plant extracts and substances; • Isolation and identification of anaerobic cellulose-degrading and hydrogen-producing bacteria from a laboratory bioreactor; Isolation of genomic bacterial DNA; Vertical DNA electrophoresis in agarose gel; Polymerase chain reaction (PCR) as a conventional end-of-reaction analysis and in real time with quantitative PCR based on TaqMan probes and absolute analysis by standard line; Gene expression; Preparation of plant extracts using phytochemical methods; Isolation, purification and identification of biologically active substances using chromatographic and spectral methods; Determination of water quality using microbiological and molecular biological approaches, physicochemical indicators and by analysis of the species composition of macrozoobenthos communities.

3.2. Characteristics of published scientific results.

Chief Assistant Professor Dr. Lyudmila Dimitrova is a co-author in a total of 38 scientific publications. Of these, 24 have an impact factor (IF: 84.068). She has achieved an H-factor of 10 (Scopus) and an H-factor of 12 (Google Scholar).

The total number of scientific papers submitted for participation in this competition is 20, but 19 are reviewed, as follows: • **Publications in indexed and refereed journals, included under indicator B.** Habilitation work – scientific publications in issues that are refereed and indexed in WoS/Scopus – 7 pcs. • **Publications in indexed and refereed journals, included under indicator D,** outside the habilitation work – 12 pcs. Thirteen articles have been submitted, but the next publication must be dropped from them: „Trochopoulos, A. G., Ilieva, Y., Kroumov, A. D., **Dimitrova, L. L.,** Pencheva-El Tibi, I., Philipov, S., ... & Zaharieva, M. M. (2022). Micellar curcumin substantially increases the antineoplastic activity of the alkylphosphocholine erufosine against TWIST1 positive utaneous T cell lymphoma cell lines. Pharmaceutics, 14(12), 2688. IF:6.525“. She is in a co-authorships with a member of the Scientific Jury for this competition. According to the LDASRB and the Regulations for its application, this is permissible for a member of the jury who does not prepare a review, i.e. there is no legal violation of the procedure. However, due to an additional requirement in the LDASRB and the Regulations for its application of the Institute of Microbiology - BAS, this is not recommended. For this reason, this article is not reviewed and the points it carries (Q1=25) are not taken into account. This does not harm the candidate, who many times exceeds the minimum criteria for indicator D, as well as for other indicators.

In six of the publications, she is a lead author, and in two of them she shares this with Y. Ilieva.

Ch. Asst. Prof. L. Dimitrova has 16 more publications outside of this competition, four of which have a total IF: 12.974, one - with SJR:0.115 and two others, included in the dissertation work for the acquisition of the ESD "Doctor" with a total IF: 2.605, Q2, in which she is the lead author.

The table presents in a comparative aspect the minimum required points by the groups of indicators for occupying the position of Associate Professor in the field of higher education Natural Sciences under the LDASRB, Professional Direction 4.3. Biological Sciences, Regulations of the Bulgarian Academy of Sciences and those obtained by the candidate in this competition:

Group of indicators	**Contents**	**Associate Professor**	
		Minimum required points	**Presented by the candidate**
A	Indicator 1	50	50
B	Indicator 2	-	-
C	Indicators 3 or 4	100	140
D	Sum of indicators 5 to 10	220	254
E	Sum of points in indicator 11	60	1066
F	Sum of indicators from 12 to the end	-	324.1
Total points		**430**	**1834,1**

According to indicator **A**, Chief Assistant Professor Dr. Dimitrova received the necessary **50** points for a defended dissertation for the award of the ESD "Doctor" on the topic "Biological activity of extracts and compounds isolated from *Geum urbanum* L.", Diploma No. 001170/25.07.2019. An abstract is attached.

Under indicator **B,** no points are required for this academic position.

Under the next indicator **C,** seven publications are presented as Habilitation work - scientific publications in issues referenced and indexed in WoS/Scopus, of which three in Q1=75 points, one in Q2 = 20 points and three in Q3= 45 points, total: **140** points.

Under group of indicators **D,** Chief Asst. dr. Dimitrova has a total of **254** points, which exceeds the required 220 points, for 12 scientific publications in issues referenced and indexed in world-renowned databases with scientific information (Web of Science and Scopus), outside the habilitation work, of which seven in Q1=200 points, two in Q2=40 points, one x Q3=15 and two in Q4 = 24 points.

By group of indicators **E** (Citations or reviews in scientific publications, referenced and indexed in WoS/Scopus or in monographs and collective volumes) Dr. Dimitrova received a total of **1066** points from **491** citations x 2 = 982 points and **42** reviews x 2 = 84 points, which also significantly exceeds the required 60 points.

By the last indicator **F,** no points are required for this academic position, but Dr. Dimitrova has a total of **324.1** points from participation in 26 projects x 10 = 260 points, management of three projects x 20 = 60 points and from attracted funds for projects managed by her - a total of 20,500 BGN (1 point for every 5,000 BGN) = 4.1 points.

Dr. Dimitrova also exceeds the additional criteria for the development of the academic staff at the Institute of Microbiology, which can be seen from the table:

Academic position	**Number of publications in journals with IF/SGR**	**Citations**	**IF**	**H-factor**	**Additional requirements**
Associate Professor	20 (after "doctor"), in 5 of them 1st author or corresponding author	100	20	5	Participation in 3 projects
Number of points for Ch. Assist. Prof. Dr. Lyudmila Dimitrova	**24** (after "doctor"), in 6 of them 1st author	Sonix: **491** Scopus: **408**	**79,468**	**10**	**26**

The presented data clearly shows that according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, theRegulations for the Implementation of the LDASRB and the Regulations on the Terms and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at the Institute of Microbiology-BAS, **the candidate not only meets the minimum national requirements for scientific activity, set out in the Regulations for the Implementation of the LDASRB for the professional field of the competition, but also significantly exceeds them. She has a total of 1834.1 points, accompanied by the relevant evidence, with a minimum requirement of 430 points** for the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences.

3.3. Reflection of the candidate's scientific activity in the literature (citability).

The large number (total **491,** excluding self-citations) of established citations of 24 scientific publications of Chief Assistant Professor Dr. Dimitrova testifies to the relevance and significance of her scientific works and their broad impact in literature in our country and abroad..

3.4. Contributions to the candidate's work (scientific, applied scientific, applied).

The scientific contributions in the presented works of Chief Asst. Prof. Dr. Dimitrova are in the field of ecology, microbiology, molecular biology and biotechnology, with a focus on the study of the antimicrobial activity of biologically active substances and their toxicological profile.

The following main contributions arise from the publications submitted for participation in the competition:

• Antineoplastic, antiviral and *in vivo* toxicological evaluation of extracts from *Geum urbanum* L. The cytotoxic profile of methanolic, petroleum ether, ethyl acetate (EA), butanolic, aqueous and 20% ethanolic extracts from aerial parts and roots of *G. urbanum* L. on tumorigenic and non-tumorigenic cell lines had been determined. For the first time, the chemical composition of the EA extract from aerial parts had been studied by high-performance liquid chromatography and mass spectrometry (UHPLC–HRMS), as well as its toxicological profile *in vivo*.

• Induction of apoptosis and suppression of "oncogenic" superoxide in Burkitt lymphoma cells. Butanol extracts showed a better cytotoxic profile against Burkitt lymphoma compared to EA extracts from aerial parts and roots, inducing cell apoptosis and suppressing "oncogenic" superoxide.

• Phenotypic inhibition of the quorum sensing system of *Pseudomonas aeruginosa* after treatment with EA extracts from G. urbanum L. At ¼ sub-minimal inhibitory concentration, EA extract from roots suppressed biofilm formation by about 84%, while that from aerial parts – by about 62%. This extract at the same concentration completely inhibited the motility of *P. aeruginosa*. The extracts completely suppressed the expression of the Las/RhI quorum sensing system, suggesting that the phenotypic manifestations are influenced by the third PQS quorum sensing system. The two EA extracts exhibited strong redox-modulating capacity, with the root extract inhibiting α-glucosidase, which has a key role in glucose absorption in the gastrointestinal tract.

• Acute and subacute toxicity of EA extract from aerial parts of *G. urbanum* L. Oral administration of EA extract in tenfold higher doses than *in vitro* results did not affect the behavior and weight of animals, hematological parameters, oxidative stress in brain tissue and blood plasma, there were no significant histopathological changes in the spleen and Peyer's patches, and no death was recorded. This makes the studied extract a suitable candidate for use as a nutritional supplement in various infectious diseases.

• An innovative approach in "green" chemistry for propolis extraction using natural deep eutectic solvents (NDES). The combination of citric acid and 1,2-propanediol extracts total phenols and total flavones and flavonols to the greatest extent. This extract has shown high activity against *Staphylococcus aureus*.

• Biological activity of propolis extracts obtained with PDER. The extraction conditions with PDER had been optimized and the chemical composition of the obtained extracts was studied by gas chromatography-mass spectrometry (GC-MS). PDER extracts showed higher antimicrobial activity compared to 70% ethanol extracts. Combinations of PDER, such as 1) D/L-lactic acid and 1,2-propanediol, 2) choline chloride and 1,2-propanediol and 3) choline chloride, 1,2-propanediol and water, have low to negligible cytotoxicity. The lowest genotoxicity had been shown by the combination of PDER choline chloride and 1,2-propanediol, as well as its extract, and the highest - by the combination of PDER D/L-lactic acid and fructose, incl. and its extract.

• Extraction of plantain (*Plantago major*) and buckthorn (*Sideritis scardica*) with PDER. These extracts of both plants, obtained with the combination of choline chloride and glycerol or citric acid and 1,2-propanediol, show higher antimicrobial activity against some potential pathogens, low toxicity and genotoxicity, compared to 70% ethanol extracts.

3.5. Visions for future research work.

Ch. Assist. Prof. Dr. Lyudmila Dimitrova has a clear vision for her future research career, intending to continue and deepen it with the following current and multifaceted approaches:

• **Phytochemical and ecological** – development of PDER techniques suitable for the extraction of biologically active substances by groups (flavonoids, tannins, etc.) from *G. urbanum* L, other species of the genus *Geum*, as well as from various plant objects. This innovative approach is safe for personnel during work and is harmless to the environment (the research will be carried out in collaboration with the Laboratory of Chemistry of Natural Substances, IGIC – BAS).

• **Microbiological, molecular biological and genetic** – it is planned to determine the antimicrobial activity of all obtained extracts (minimum inhibitory and bactericidal concentration, inhibition of biofilm formation, suppression of pyocyanin synthesis, motility and protease activity). The mechanism of action of the most promising ones will be determined by monitoring the expression of genes responsible for adhesion, biofilm formation, quorum sensing, cellulose degradation, etc. with RT-PCR and real-time PCR. An important perspective is the search for changes in the bacterial genome and bioinformatics analysis. In the same way, various substances of natural or synthetic origin will be tested.

• **Antioxidant and redox-modulating** – PDER extracts or substances of natural or synthetic origin that have shown antimicrobial activity will be investigated for their ability to scavenge free radicals (in collaboration with the Laboratory "Modifiers of Biological Response and Pathogenesis of Viral Infections", IMikB – BAS).

• **Cytotoxic** – the cytotoxic profile of the PDER extracts or substances of natural or synthetic origin that have shown good antimicrobial and antioxidant activity will be evaluated on normal cell lines. Promising PDER extracts or substances will be selected and their antineoplastic and antiviral potential will be further evaluated (in collaboration with the Lab. "Cytotoxicity and Signal Transduction" and the Department of Virology, IMikB – BAS).

• ***In vivo*** – PDER extracts or substances of natural or synthetic origin, which have shown strong biological activity and a favorable cytotoxic profile, will be tested in animal models. The candidate in the competition has experience in determining acute and subacute toxicity in mice, skin sensitization in guinea pigs and skin irritation in rabbits, while complying with all protocols for safe work and humane treatment of laboratory animals. The substances or extracts will be tested for their effect on the healing of infected wounds in skin infection of mice, reproduced by the punch biopsy method.

• **Biotechnological** – search for non-pathogenic cellulolytic bacteria that would find application in biotechnology for the utilization of lignocellulosic waste.

• **Scientifically applied** – the developed modern methods and approaches will be implemented in laboratory practice for the study of various natural or synthetic objects and training of students and doctoral candidates. The results obtained will serve as a foundation for writing and submitting new projects and/or utility models, as well as for seeking cooperation with companies.

3. 6. Other scientific and publication activities of the candidate.

L. Dimitrova's scientific work as a young microbiologist has been honored with a number of **awards,** the most significant of which are the awards of the "Acad. Prof. Dr. Stefan Angelov" Foundation for the best work of a young microbiologist for 2017, 2019, 2020 and 2023. In 2022, she was among the nominees for the "Pythagoras" Award for a young scientist in the field of Life Sciences and Medicine.

Over the past 5 years (2021 - 2025), Chief Asst. Prof. Dr. Lyudmila Dimitrova has participated with **13 poster presentations in 8 scientific forums** such as the 7th International Conference with Youth Scientific Session and MELiSSA Summer University, Ecological Engineering and Environment Protection, Varna, Bulgaria, 30.09.2021 - 03.10.2021; 14th Scientific conference of the Bulgarian focal point of EFSA Risk Assessment Center on Food Chain 'Sustainable science for safety food'. Bulgaria (online) - 27.10.2021; Kliment's Days 2021, Sofia, Bulgaria; 120 years of the National Diagnostic Research Veterinary Institute and 140 years of the birth of its patron Prof. Dr. Georgi Pavlov, Sofia, Bulgaria, 21.10.2021; 5th International Conference on Natural Products Utilization: From Plants to Pharmacy Shelf, Sts. Constantine and Helena, Bulgaria, 30.05.2023 - 02.06.2023; International Conference "Clean Nature for Health, Velingrad, Bulgaria, 10.09.2023, Eighth International Conference ECOLOGICAL ENGINEERING AND ENVIRONMENT PROTECTION with Youth Scientific Session, Velingrad, Bulgaria, 03.10.2024 - 06.10.2024, The 4th International Electronic Conference on Antibiotics. Challenges and Strategies for the Antibiotic Resistance Crisis, Online, 21.05.2025 - 05/23/2025

She has also participated with **8 oral presentations in 7 scientific forums:** Tradition and Modernity in Veterinary Medicine 2021, Sofia (online), Bulgaria, 16.04.2021 – 18.04.2021; 14th Scientific conference of the Bulgarian focal point of EFSA Risk Assessment Center on Food

Chain 'Sustainable science for safety food'. Bulgaria (online), 27.10.2021; Humboldt-Kolleg, Arbanasi, Bulgaria, 30.06.2022 - 03.07.2022; XVth Congress of the Bulgarian Microbiologists with International Participation, Koprivshtitsa, Bulgaria, 05.10.2022 - 08.10.2022; International Scientific Conference "Tradition and Modernity in Veterinary Medicine", Yundola, Bulgaria, 28.04.2023 - 30.04.2023; International Conference "Clean Nature for Health, Velingrad, Bulgaria, 10.09.2023 - 13.09.2023 and 2nd International Applied Scientific Conference Medicinal, Aromatic and Edible Plants (MAEPs) and their by-products: Utilization and valorization for a sustainable and circular economy, Plovdiv, Bulgaria, 16.05.2025 - 17.05.2025

4. Assessment of the candidate's personal contribution.

The personal contribution to the scientific production of Chief Asst. Prof. Dr. Lyudmila Dimitrova is evident from the authorship of the publications submitted for participation in this competition. In six articles she is the lead author, in two of them she shares this with Y. Ilieva. In another two she is the second author, and in five – the third one.

5. Evaluation of the candidate's teaching activities.

In 2024-2025, Dr. Dimitrova has conducted a course of 60 practical exercises in Microbiology at the Faculty of Medicine of Sofia University with 3rd year Medical students, although no official document was attached to this. This activity presents her as an experienced teacher..

6. Critical notes and recommendations.

I would recommend that Senior Assistant Professor Dr. L. Dimitrova continue to work actively and dedicatedly as a scientist, to participate in and lead research projects, as well as to publish her results in scientific issues, refereed and indexed in world-renowned databases, more often as a lead author.

8. Conclusion.

Based on the information presented above, I propose that **the candidate, Chief Assistant Professor Dr. Lyudmila Lyudmilova Dimitrova to occupy the academic position "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Microbiology" in the Department of "Infectious Microbiology", Laboratory "Bacterial Virulence, Resistance and New Antimicrobial Agents" of the Institute of Microbiology "Acad. Stefan Angelov" at the Bulgarian Academy of Sciences.**

15.11.2025 г. Reviewer's signature:

Sofia (Prof. D.Sc. Teodora Popova)