

OPINION

by Prof. Dr. Denitsa Yancheva Pantaleeva, Institute of Organic Chemistry with Centre of Phytochemistry, BAS

on the materials submitted for participation in the competition

for the academic position of ‘Professor’

at the Institute of Organic Chemistry with Center of Phytochemistry (IOCCF), BAS

by field of higher education

4. “Natural Sciences, Mathematics and Informatics, professional field”,

professional field “Chemical Sciences” - 4.2, scientific specialty “Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances” for the needs of Lab. CBPE

1. General presentation of the procedure and the candidate

In the competition for ‘professor’, announced in the State Gazette, no. 104 of 10.12.2024 and on the internet page of the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS, as the only participating candidate is Assoc. Prof. Dr. Lyudmila Velkova. The set of materials on paper, submitted by her, is in accordance with the Regulations for the Development of the Academic Staff of IOCCP, and meets the criteria of the IOCCP-BAS for holding the academic position of Professor.

For the competition the candidate Assoc. Prof. Dr. L. Velkova has submitted a total of 40 scientific papers, 38 scientific articles and 2 collective monographs, and a list of 2 scientific and applied developments (utility models). All submitted materials are accepted for review in regard to the competition and 35 research projects are also considered in the final evaluation. For the purposes of this competition, 28 research papers are not reviewed (related to previous dissertation defense and competition for the academic position of Associate Professor). For the competition, the candidate has submitted 265 citations of her scientific works, with the total citation rate of Assoc. Prof. Dr. L. Velkova reflected by an h-factor of 12 (based on the data in Scopus, after excluding the citations of all co-authors).

The distribution of scientific papers among the respective Q factors is as follows - 5 publications in Q1 journals and 1 publication in a Q2 journal (presented for indicator C); respectively 5 publications in Q1 journals, 6 publications in Q2 journals, 8 publications in Q3 journals and 3 publications in Q4 journals (presented for indicator D). Documentation of participation in 2 recognized utility model applications, both on their utility and on the economic impact obtained, is also provided. Assoc. Prof. Dr. Lyudmila Velkova has also submitted a habilitation thesis (extended habilitation statement of contributions) in the volume of 16 pages, evidence of participation in scientific projects and scientific forums on the subject of the competition.

The documents are clearly and systematically formatted and support all necessary categories for the evaluation of the candidate's activity in the competition.

Assoc. Prof. Dr. Velkova has completed her higher education at the Faculty of Chemistry, Sofia University. Further she continued her education as a PhD student in the laboratory CBPE of the Institute of Organic Chemistry with the Centre of Phytochemistry - BAS (in the period 2009-2012), under the scientific supervision of Prof. Dr. P. Dolashka. The entire previous scientific career of Assoc. Velkova has passed at the Institute of Organic Chemistry with the Centre of Phytochemistry, where she has successively held the positions of chemist, assistant, senior assistant and associate professor (associate professor - since 2019).

According to my personal impressions, Assoc. Prof. Dr. L. Velkova is an active researcher, participating in a number of interdisciplinary teams for the implementation of scientific projects and scientific and applied developments in the field of bioorganic chemistry, who also devotes a significant part of her time to the training of younger colleagues.

2. General description of the candidate's activities

The scientific papers submitted for the competition show that the research interests of Assoc. Prof. Dr. Velkova's research interests are in the field of bioorganic chemistry and chemistry of natural and physiologically active substances.

The candidate's research efforts have been directed in four areas: (i) identification and characterization of biocomponents (peptides, proteins and glycoproteins) in *Gastropod mucus* and haemolymph and study of their antimicrobial activity; (ii) study of antitumour potential of components of *Gastropod* haemolymph and mucus; (iii) elucidation of the mechanism of action of snail biocomponents established by proteome analysis of two-dimensional gel electrophoresis (2D-PAGE); (iv) characterization of other bioactive components from natural sources. The main contributions of Assoc. Prof. Dr. Velkova in the conducted multidisciplinary research are related to the development of new approaches for isolation and characterization of active components in complex multicomponent biological mixtures, clarification of the relationship between structure, function and biological activity, establishment of the mechanism of action of active components. The identification and characterization of biocomponents in snail mucus and hemolymph, the study of their antimicrobial, antitumor properties and their mechanism of action are discussed in the extended habilitation statement of contributions of Assoc. Prof. Dr. Velkova.

With regard to the isolation and characterization of biocomponents, the most significant contributions include the development of a methodology for the characterization of peptides from the mucus of *C. aspersum* (garden snail); building a hypothesis for the spontaneous formation of peptide nanostructures (clusters) in the mucus of *C. aspersum*. based on *in silico* simulations and its experimental support by means of the developed methodology based on UV-vis spectroscopic and fluorescence studies and *in vitro* assays of peptide antibacterial activity; development of a methodology based on a bioinformatics approach to elucidate the relationship between structure and antimicrobial activity, and demonstration of the antibacterial potential of peptide fractions isolated from snail mucus against *Pseudomonas aureofaciens* AP9, *Brevillius laterosporus* BT271 and *Escherichia coli* NBIMCC 878 (results for one of the fractions are included in a utility model application); identification of proteins in the hemolymph composition of the sea snail *Rapana venosa* and demonstration of the antibacterial potential of the protein fraction; demonstration of the fungicidal effect of two gastropod fractions against fungal strains resistant to nystatin and amphotericin - *Aspergillus niger* and *Penicillium griseofulvum*.

The results of biological studies on the antiproliferative activity of components of *Gastropod* haemolymph and mucus have led to the elucidation of the promising potential of different isoforms and functional units of hemocyanins from *H. lucorum*, *H. aspersa* and *R. venosa*, as well as some fractions from the hemolymph of *R. venosa* and the mucus of *C. aspersum* against cancer cell lines of diverse origin and different genetic profiles (breast cancer, human bladder carcinoma). Novel approaches have been applied in these studies, including combinations of different methods for optimization, isolation and purification of native hemocyanins and their isoforms, characterization by electrophoretic and MALDI TOF/MS analyses, detection of antiproliferative changes in tumor cells after treatment with different forms of hemocyanins, and clarification of the role of specific oligosaccharide structures located on top of the functional and structural subunits of hemocyanins.

The identification of a broad set of proteins in the T24 human bladder carcinoma cell line altering their expression after treatment with the N-glycosylated functional unit β c HIH h of *H.*

lucorum hemocyanin; elucidation of the mechanism of action of snail biocomponents; elucidating the mechanism of the preventive action of a standardized mucus extract of *C. aspersum* (EC), enriched in a fraction with MT above 20 kDa, on Alzheimer-type dementia in rats, based on the specification of protein expression changes in the rat cortex, the identification of a number of proteins with altered regulation and those related to memory and cognitive functions, can be highlighted as major contributions in the third area of research, established by proteome analysis of 2D gel electrophoresis (2D-PAGE).

In the characterization of other bioactive components from natural sources, the primary structures of 17 novel peptides were identified in the fraction with MT below 1 kDa from the hemolymph of the garden snail *H. lucorum* on the basis of *de novo* sequencing, comprising mostly cationic amphipathic structures with hydrophobic surfaces that differ significantly from peptides with potential antioxidant properties found in the mucus of *C. aspersum*; the antioxidant potential of peptides and secondary metabolites from the hemolymph of the garden snail *H. lucorum* was established and shown to be mainly due to peptides with low molecular mass and specific amino acid sequences, predominately including Leu, Val, Phe, His, Pro, Lys and Tyr residues; a methodology for isolation, purification and characterization of cyclolipopeptides from cell-free supernatants of *B. velezensis* R22 was developed and the major active compounds in the extract of *B. velezensis* R22 and their primary structures were determined.

The contributions described above have both fundamental scientific character and significant potential for practical application in medical practice, which is supported by the two submitted utility model applications.

The review of the submission materials demonstrates reliably the major role of Assoc. Prof. Dr. L. Velkova's role in the research, as she is the lead author or corresponding author in a significant number of the publications and conference papers. The leadership of scientific projects in the field of the competition and related to the submitted scientific publications, scientific and applied developments are also good evidence of the active and leading participation of Assoc. Velkova.

3. Critical comments and recommendations

I have no critical comments on the submitted materials.

CONCLUSION

The documents and materials submitted by Assoc. Prof. Dr. Lyudmila Georgieva Velkova from the Institute of Organic Chemistry with Centre of Phytochemistry, BAS, meet all the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the National regulations for its implementation, and the respective Regulations for its implementation of BAS and IOCCP-BAS.

The candidate in the competition has submitted a significant number of scientific works published after the materials used in the defense of her PhD thesis and the preceding competition for the academic position of Associate Professor. The candidate's works contain original scientific and applied contributions that have received international recognition, all of them have been published in journals of international academic publishers. Her scientific works have also practical applicability. The scientific qualifications of Assoc. Prof. Dr. Lyudmila Georgieva Velkova are unquestionable.

The results achieved by Assoc. Prof. Dr. Lyudmila Georgieva Velkova in the scientific research activities fully comply with the specific requirements of the Regulations of the IOHCF-BAS for implementation of the Law for the Development of Academic Staff in the Republic of Bulgaria.

Therefore, I confidently recommend to the members of the Scientific Jury and the Scientific Council of IOCCP-BAS to vote “YES” for the election of Assoc. Prof. Dr. Lyudmila Georgieva Velkova to the academic position of “Professor” in the field 4. “Natural sciences, mathematics and informatics, professional field”, professional field “Chemical sciences” - 4.2, scientific specialty “Bioorganic chemistry, chemistry of natural and physiologically active substances”.

22.04.2025 г.

Jury member:

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(Prof. Dr. Denitsa Pantaleeva)