

REVIEW

in a competition for the academic position "professor", professional area 4.3. Biological Sciences (Cell Biology), field of higher education 4. "Natural Sciences, Mathematics and Informatics", announced in SG no. 32/09/04/2024 for the needs of the Faculty of Biology at Sofia University "St. Kliment Ohridski".

Reviewer: Academician Roumen Pankov, appointed as a member of the Scientific Jury according to order No. RD-38-258/29.05.2024 of the Rector of Sofia University "St. Kliment Ohridski".

At the announced competition, the only candidate who submitted documents was associate professor Dr. Tanya Ivanova Topouzova-Hristova, from the Department of Cell and Developmental Biology of the Faculty of Biology at the SU «St. Kliment Ohridski». According to the document presented by the "Human Resources" department, Associate Professor Topouzova-Hristova has experience in the specialty for more than 28 years, which fully meets the requirements set forth in the ZRASRB and the Regulations for its application. The materials submitted by the applicant have been prepared and completed in accordance with the legal requirements.

Biographical data for the candidate

Assoc. Prof. Tanya Topouzova-Hristova was born in 1969. She graduated from the Faculty of Biology of Sofia University "St. Kliment Ohridski" with a specialization in "Cell and Developmental Biology" in 1994. She began her professional career as a biology teacher, and in 1996, after a competition, she was appointed an assistant professor in the Department of "Cell and Developmental Biology" (then "Cytology, Histology and Embryology") of BF at SU, where she successively passed through the positions of senior (2000) and chief assistant professor (2004). In 2008, she successfully defended her dissertation on the topic "Influence of halogenated inhalation anesthetics on the integrity and reparative abilities of lung cells" as a free doctoral student. After a competition held in 2014, Dr. Topouzova-Hristova was elected as an associate professor at the same department where she has been working until now. Since 2020, she is the head of the department.

Prof. Topouzova-Hristova specialized for 6 months at the University of Seville, Spain.

Analysis of scientific production and scientometric data

The total scientific output presented by Assoc. Prof. Topouzova-Hristova includes 74 publications, 9 textbooks and 62 participations with oral presentations and posters in national and international scientific forums. Of all scientific articles, 41 were published in international journals with IF and/or Q rank, among them prestigious journals such as *Pharmaceutics* (IF 6.5), *Polymers* (IF 4.3), *Metabolites* (IF 4.1), *Colloids and Surfaces B: Biointerfaces* (IF 4.3) etc. The total impact factor of the presented scientific papers is over 88, and in the reference from the "Authors" system, 238 titles without self-citations are indicated for the citations. According to the data from Scopus, Assoc. Prof. Topouzova-Hristova's Hirsch index is 11.

For her participation in the current competition, Assoc. Prof. Topouzova-Hristova presented 25 scientific publications and 9 textbooks that have not been reviewed in previous competitions for the award of scientific degrees or academic positions. Scientific articles on this competition make up 33.7% of her entire scientific output, and the analysis of publication activity over the years shows that after her habilitation in 2014, she published an average of 2.5 articles per year. This certainly demonstrates an active research, especially for a university professor who is also

engaged in significant lecturing and administrative work. Of the articles in the current procedure, 18 were published in journals with an impact factor (total IF 57.6), with 9 (50%) in journals with an impact rank Q1, 7 (38.9%) - in Q2, and two (11, 1%) - in Q3. This distribution well illustrates not only the intensity, but also the high quality of the scientific research conducted by Assoc. Prof. Topouzova-Hristova.

The scientific production presented and the scientometric data achieved fully correspond, and in many of the indicators, exceed the minimum requirements for awarding the academic position "professor", defined in the Regulations for the terms and conditions for acquiring scientific degrees and for holding academic positions at SU. Data for meeting the requirements by indicators are presented as follows:

- Indicators from group A: dissertation work - 50 points.
- Indicators from group B: habilitation thesis - 140 points out of the required 100 points.
- Indicators from group D: publications in journals Q1 – Q4 - 240 points out of the required 200 points.

- Indicators from group D: cited works – 408 points out of the required 100 points. Although the submitted documentation notes 166 Scopus citations, the check shows 204 citations, excluding self-citations, which gives me the reason to indicate as correct 408 points on this indicator.

- Indicators from group E: with the required 150 points, Assoc. Prof. Topouzova-Hristova provided data on 281.8 points achieved. The management of two (40 points) and participation in 13 (130 points) national projects bring a total of 170 points, and the attracted over BGN 158,000 from projects add another 31.8 points to this indicator. Associate Professor Topouzova-Hristova is the supervisor of one successfully defended doctoral student and is currently supervising another one (50 points), and the teaching aids add another 30 points to the group E indicators.

In total, according to the scoring rules, Associate Professor Topouzova-Hristova achieved 1119.8 points, which is almost twice as many as the minimum criteria for the academic position "professor" of 600 points.

Analysis of scientific contributions

Assoc. Prof. Topouzova-Hristova's scientific interests and published scientific results are entirely in the field of the announced competition and are focused in two main areas:

- Characterization of the effects of various secondary metabolites of plant origin on pro- and eukaryotic cells in order to discover potential phytopharmacological preparations and
- Creating and characterizing the action of new nanosized drug carriers.

The results presented in the hitherto unreviewed publications are summarized in groups B and G, according to Appendix 1 of the PPZRASRB. I will examine the presented articles as they are systematized by the candidate, and in a summarized form will present the most important scientific achievements, from which the scientific contributions follow.

In group "B" 12 publications are presented, of which 7 are in journals with an impact factor (the total IF of these journals is 23.1). One of these articles is in a journal from Q1, five - from Q2 and one - from Q3. The articles present research results that belong to the first, mentioned above, direction and actually represent a continuation of Assoc. Prof. Topouzova-Hristovna's scientific interests, dating back to the time when her dissertation was being developed. In the presented studies, she focused on the biological effects that extracts of famous Bulgarian medicinal plants can have. The effects of extracts from the endemic resurgent sylvatic plant Orpheus flower (*Haberlea rhodopensis*), white dead nettle (*Lamium album L.*), nine species of plants from the

genus *Inula* (Oman) and rose oil (*Rosa damascene* Mill.) were studied. The results obtained can be summarized as follows:

- The phenyl glycoside myconoside, a specific secondary metabolite from Orpheus flower, can directly affect the degree of lipid ordering in the membranes of cancer and non-cancer cells, leading to changes in their vitality, organization of the actin cytoskeleton and proliferative activity. This observation was also confirmed on biomimetic membranes and offers an opportunity to explain the molecular mechanism by which myconoside exerts the established biological effects;

- Through the study of secondary metabolites of white dead nettle isolated from plants grown in their natural habitats, in vitro propagated plants and after their subsequent cultivation in a natural environment, have been shown that extracts from wild and in vitro cultivated plants have different activities, such as despite their poorer composition, secondary metabolites from in vitro cultivated plants have better antitumor activity;

- After studying the main groups of secondary metabolites of nine species of oman and mainly of chlorogenic (5-CQA), 1,5-, 3,5-, 4,5- and 3,4-dicaffeoylquinic (DCQA) acids, a total fraction of flavonoids, flavonoid glycosides, phenolic acids and sesquiterpene lactones, the critical concentrations of cytotoxicity and in vitro biological activity exerted on various cancer and non-cancer cell lines have been established. These studies allowed the authors to determine the appropriate plant species and groups of metabolites with antitumor potential;

- A detailed phytochemical analysis of two rose oil extracts was carried out and 14 kaempferol glycosides, 12 quercetin glycosides, 4 phenolic acids and their esters, 4 galloyl glycosides, 7 ellagitannins and quinic acid were found. The ethyl acetate extract was shown to be richer in total phenolic and total flavonoid compounds and showed better antioxidant activity compared to the total dry extract. An interesting result of these studies is the low toxicity found for both extracts on normal human skin fibroblasts, combined with significant antibacterial activity. The results allow these extracts to be identified as potential candidates for inclusion in cosmetic and medicinal (antibacterial) formulations for skin application.

The papers from the second group - group G, with which Assoc. Prof. Topouzova-Hristova participats in the current competition, include 13 scientific articles, of which 11 were published in journals with an impact factor (total IF 34.5) and two - in journals without an IF. Of all articles with IF, 8 (73%) were published in journals belonging to Q1, 2 (18%) were from Q2 and one (9%) – from Q3. The articles of this group summarize research results, which can be provisionally divided into two groups.

The first group includes articles related to studies on natural organic compounds, but unlike studies on plant extracts, is mainly focused on elucidating the molecular mechanisms causing the biological effects. Logically, these studies have focused on the cell membrane, as the first cell structure interacting with the studied preparations:

- It has been found that in in vivo-like conditions, sequestration of activated ERK1/2 in lipid rafts can regulate cell proliferation;

- It has been shown that the internalization of alkylphospholipids through the raft-domains of membranes leads to the influence of lipid-dependent signaling pathways in a cell-specific manner;

- The manifestation of the different cellular response - cell death or high genotoxicity depends on the different involvement of the enzymatic and non-enzymatic part of vipoxin (phospholipase A2 from snake venom);

- Protocols for microscopic evaluation of lipid domains in live cells based on Laurdan and Di-4-ANEPPDHQ were optimized, showing that Di-4-ANEPPDHQ is more suitable for observation with conventional fluorescence and confocal microscopes.

The rest of the publications in group D summarize the results of the second main direction of research of Associate Professor Topouzova-Hristova, namely - Creation and characterization of the action of new nano-sized drug carriers with an emphasis on the mechanisms of internalization and the dynamics of release of the biologically-active material in the cells. The main contributions of this body of research are:

- Homogeneous coamorphous microsphere type structures have a very good drug loading capacity and offer a "controlled" desorption profile.

- Collagen-titanate nanocomposites have broad-spectrum antimicrobial activity, as well as cell-specific cytotoxicity for eukaryotic cells depending on the concentration of ZnTiO₃. Collagen-RGO nanocomposites are active to Gram-positive microorganisms and *Candida lusitanae*, but non-toxic to Gram-negative and human cells, making them a promising antimicrobial biomaterial.

- Comb-like polyethylenimines successfully condense linear and plasmid DNA into nanosized polyplex particles that can be used for transfection of eukaryotic cells. The mode of internalization of the particles and the efficiency of transfection depend on the topology of the polymer chain.

- Nanocapsules of amphiphilic triblock copolymer cationic micelles with DNA have very low cytotoxicity and insignificant damage to the cell membranes of human cells, which makes them suitable for carrying medicinal preparations. Loaded with AgNO₃, these micelles successfully destroy bacterial biofilms and demonstrate strong antibacterial activity.

- Mixed polymer micelles of cationic and nonionic copolymers in different ratios, alone or loaded with the antibiotic ciprofloxacin, show good antibiofilm and antibacterial activity.

- Conjugates of nucleic acids and polymer (spherical nucleic acids) have good colloidal stability, increased resistance to nucleases, low cytotoxicity and increased cellular internalization.

Teaching and administrative activity

As a lecturer at the university, Assoc. Prof. Topouzova-Hristova is also engaged in active teaching activities, which according to the presented documentation exceeds 450 hours of classroom employment per year. She conducts 7 lecture courses in the field of the current competition for full-time and part-time students. Of these, 5 are in bachelor's and 2 in master's degrees. A contribution to her educational activity is the co-authorship of 9 textbooks, among which the cell biology exercise manual for SU students and seven related to the biology and health education olympiads in Bulgaria. It should be noted that she has been a member of the National Commission for the Biology and Health Education Olympiad for over 20 years.

Assoc. Prof. Topouzova-Hristova was the supervisor of 14 graduate students and she supervised one doctoral student who has already defended her degree and supervises another one whose defense is planned for 2026.

The presented data characterize Assoc. Prof. Topouzova-Hristova as a lecturer with intensive teaching in the field of cell biology, covering all aspects of this activity - from the development and delivery of new lecture courses, through the writing of study aids necessary for the preparation of students, to individual work with them and with scholars showing an interest in biological sciences.

Assoc. Prof. Topouzova-Hristova is respected by her students and colleagues and enjoys the name of a highly qualified and erudite teacher. She was the head of the Department of Cell and Developmental Biology (2020-2024), and most recently she was also elected as deputy dean of academic activities - "master" and accreditation of the Faculty of Biology.

Conclusion

As a long-time member of the Department of Cell and Developmental Biology, I have the pleasure of personally knowing Assoc. Prof. Topouzova-Hristova and for years witnessing her successes as a researcher and teacher. She is an established and sought-after specialist with high professional qualifications and active research. Her scientific output is significant in volume and quality and, as noted, exceeds the requirements for the award of the academic position of "professor" referred to in the normative documents. There is extensive experience in leadership and team work, training of doctoral students and graduates, competencies and skills for shaping concepts and realization of scientific publications and projects. This gives me reason to confidently give my positive assessment and to recommend to the Scientific Jury to elect Associate Professor Dr. Tanya Ivanova Topouzova-Hristova as "Professor" in professional direction 4.3. Biological Sciences, with a scientific specialty "Cell Biology".

Sofia 07/07/2024

Reviewer:

Acad. Roumen Pankov