OPINION
of Associate Prof. Reneta Slavova Gevrenova, PhD
Faculty of Pharmacy, Medical University-Sofia
on the materials, presented for participation in the competition
for obtaining the Academic degree **"Associate professor"**
at the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS

Higher education area 4. "Natural sciences, mathematics and informatics", professional field 4.2. Chemical sciences, scientific discipline "Bioorganic chemistry, chemistry of natural and physiologically active compounds"

In the procedure for "Associate professor" announced in the Bulgarian State Official Journal, 43/31.05.2019 and on the website of the IOCCP, BAS, as a candidate participates Senior Assistant Professor Kalina Moneva Danova, PhD, Laboratory of "Chemistry of Natural Compounds", IOCCP, BAS.

2. General representation of the procedure and the candidate

As a participant in the announced procedure the only candidate who has submitted documents is Kalina Moneva Danova, PhD in the scientific topic "Plant physiology", Senior Assistant Professor at the Institute of Organic Chemistry with Centre of Phytochemistry, BAS.

The materials presented by Dr Kalina Danova as hard copy and in electronic form are in compliance with the Regulations for Academic staff development at IOCCP and comply with the criteria of IOCCP, BAS for obtaining the Academic degree "Associate professor".

Dr Danova participates in the competition with overall 21 scientific works - 18 publications and 3 book chapters. The habilitation work includes 7 publications (group "B" of the minimal requirements of IOCCP, BAS) and is accompanied by a list of the habilitation contributions. Group "Г" includes 11 publications and three book chapters and the group of "other publications of the candidate" includes 25 works. A list of 14 scientific research projects has been presented. Dr Danova is the leader in 6, coordinator of 1 and participant in 7 of them.

21 works, which are aside from the PhD thesis are being considered for review and taken into consideration for the final evaluation, as well as 14 scientific research projects. The allocation of the scientific works in the B and Г groups is as follows: Q1 - 5, Q2 – 5, Q3 – 5 и Q4 – 3. In addition, documentation for the participation in 14 scientific research projects, as well as information for the financing of 6 of them has also been presented.

All lists with documents are accompanied by the full texts of the publications, certificates and information letters for the scientific projects.

3. General characteristics of the activities of the candidate

Evaluation of the scientific and scientific-applied activity of the candidate

The publications with which Dr Danova participates in the procedure, included in group B are related to *in vitro* culture establishment of the *Hypericum* species (B-Q3-3 and B-Q3-4), *Artemisia alba* (B-Q1-1, B-Q1-2, B-Q3-1 and B-Q3-2), *Clinopodium vulgare* (B-Q3-3) and *Hippophae rhamnoides* (B-Q2-1). The works are focused on optimization of cultivation media by

the application of plant growth regulators and aiming at accumulation of biologically active secondary metabolites of the chemical classes of terpenoids and phenolic compounds. In this direction, morphometric studies, characterization of the metabolic profile of extracts of the cultivated plants, as well as qualitative determination of different group of compounds has been performed.

One of the aspects of the investigations is related to the optimization of the cultivation parameters aiming at increasing the antioxidant potential of the extracts and evaluation of key enzymes in the biosynthesis of plant metabolites. Of special interests are the studies, related to the influence on the terpenoid biosynthetic pathways through affecting chloroplast architecture and the interrelations of this phenomenon with the levels of endogenous cytokinins in shoot cultures of *Artemisia alba.* Noteworthy, these studies utilize the biosynthetic potential of the plant species without performing genetic manipulations, which opens up a perspective for utilizing the obtained plant material in practice.

Another aspect of the work of Dr Danova (publications included in group Г) are classical phytochemical investigations of the plant species *Artemisia alba* (Г-Q1-3 and Г-Q2-3), *Inula britannica* (Г-Q2-1 and Г-Q2-2), *Anthemis rumelica* (Г-Q3-1), *Anthriscus cerefolium* (Г-Q4-2) related to characterization of compounds of the chemical classes of terpenoids and phenolics. Group Г also contains work on tissue culture of some *Hypericum* species (Г-Q1-1, Г-Q1-2 and Г-Q4-1), as well as a review article on the section Empedoclea of the species of the *Sideritis* genus (Г-Q2-4). Dr Danova shows high competence in the field of plant physiology, phytochemistry and plant biotechnology in three book chapters (Г-Book_Ch-1, 2 and 3) focused on the up-to-date investigations of medicinal plants, which works I would place in a separate aspect of her publication activity. They are result of scientific research performed within scientific projects. Here she shows excellent knowledge on the literature data making analytical discussions and drawing the necessary for her work conclusions. I would especially put accent on the work on the Bulgarian taxa of the *Hypericum* genus as sources of biologically active compounds.

Dr Danova participates in the procedure with 15 scientific publications which are refereed and indexed in the international databases; 11 of them are with impact factor (4 are above 2). She has presented 3 plenary lectures, was an invited lector at 2 conferences and presented 7 oral presentations. All publications and conference contributions are in English. In 8 publications she is the first author, in 2 – the second author and in the three chapters is the only author. In all publications of group B the candidate is the corresponding author. In all publications the contribution of the candidate is in the field of plant biotechnology, as well as spectrophotometric assays and preparation of extracts and fractions.

According to the presented habilitation work, the scientific contributions of Dr Danova can be considered in three main aspects:

1. Conservational aspect – *in vitro* cultures of medicinal and aromatic plants have been established and the conditions for their cultivation have been optimized. This makes it possible to maintain an *in vitro* collection of a high number of medicinal and aromatic plant species, collected from the Bulgarian flora, as well as from other regions, including some rare species (B-Q2-1, B-Q1-1, B-Q3-3, B-Q3-4, Г-Q1-1, Г-Q1-2 and Г-Q4-1). Approaches and methods of plant biotechnology have been used; the morphogenic effects of the medium parameters have been evaluated, the composition and activity of the obtained extracts have been performed. This

contribution is related to the creating of conditions for cultivation of *in vitro* plant cultures for obtaining plant biomass with desired qualities.

2. Fundamental aspect – the availability of an *in vitro* collection of medicinal and aromatic plants – producers of secondary metabolites of different chemical classes makes possible the performance of complex interdisciplinary research. Plants' growth, development and physiological status, metabolic profile and activity of extracts of the obtained biomass have been studied. This is a key moment in the creating of a scientifically-based approach for optimization of the plant productivity *in vitro* (B-Q1-1, B-Q1-2, B-Q3-1, B-Q3-2, B-Q3-4, Г-Q1-1). This contribution is related to acquiring new opportunities for the delivery of secondary metabolites with potential importance in pharmaceutical practice. A hypothesis has been formulated for the interrelations between the morphogenesis, functions of plant enzymes and content of phenolic compounds in shoot cultures (B-Q3-1, B-Q3-4). An innovative approach relates *in vitro* morphogenesis in shoot cultures and the biosynthesis of mono- and sesquiterpenes (B-Q1-1, B-Q1-2). Another hypothesis defines the correlations between the biogenesis of different terpenoid classes, bioactive cytokinins and chloroplast architecture in the plant cell of the different morphotypes (B-Q1-2).

3. Applied aspect – concrete biological *in vitro* systems have been obtained, enabling the controlled cultivation of plant material with defined qualities without the use of genetic manipulations (which thus do not fall into the restrictions of ethical and legistative restrictions for practical utilization) (B-Q1-1-2, B-Q3-1-4, Г-Q1-1). The practical application of the plant biomass is in the food and cosmetics industry, as well as in medicine. The contributory character is related to the establishment of new sources of chemical compounds with known phytopharmacological potential.

56 citations of the works of the candidate have been established, 7 of which are on publications from group B. Most cited are publications Г-Q1-1 (10 citations), Г-Q1-3 (7 citations) and Г-Q1-2 (5 citations). The citations list shows the significance of the contributions of the candidate in conservation and fundamental aspect. In this aspect, relevance within the scientific community has the study of the effect of growth regulators on the morphogenesis and terpenoid profile in *Artemisia alba* shoot cultures (B-Q1-1, B-Q1-2), as well as the investigations on the polyphenolic production and hypericin effect on stress markers in shoot cultures of *Hypericum* species (Г-Q1-1). Among the citing sources are the refereed journals Phytochemistry Reviews, Phytochemistry Letters, Analytical Methods, Industrial Crops and Products. Over half of the citations have been made after 2015. The h-factor of the candidate is 5.

The perspectives for the development of the scientific work of Dr Danova are related to continuation of the investigations of the biogenesis of phenolic compounds, endogenic hormonal regulation and photosynthetic processes in *Artemisia alba in vitro* cultures. *In vitro* systems for the production of sesquiterpene lactons of *Inula britannica* and flavonoid diglycosides in *Sideritis scardica* will be done. Investigations on root cultures of *I. britannica*, as well as non-differentiated cultures of *A. alba* in liquid media are foreseen. Some of these investigations are within the frameworks of ongoing projects.

3. Critical remarks and recommendations

I do not have any critical remarks to Dr Danova.

CONCLUSION

The documents and materials presented by Dr Kalina Danova comply with all requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for ADASRB application, the Regulations for ADASRB application in BAS, as well as Regulations of IOCCP, BAS.

The candidate in the competition has presented a considerable number of scientific publications, published after the materials used for defense of the educational-scientific degree "PhD". In the works of the candidate there are original scientific and applied contributions, which have received international recognition as a representable part of them has been published in journals and books issued by international academic publishers. Her theoretic works have practical applicability.

The results, obtained by Dr Danova in her scientific and research work completely comply with the specific requirements of the Regulations of IOCCP, BAS for ADASRB application.

After acquaintance with the presented in the competition materials and scientific works, analysis of their significance and the contained in them scientific, scientifically-applied and applied contributions, I find it relevant to give my positive evaluation and to recommend to the Scientific Jury to prepare a report – proposal to the Scientific Council of IOCCP, BAS for selection of Dr Kalina Moneva Danova for the Academic degree "Associate professor" at IOCCP, BAS in professional field 4.2. Chemical sciences, scientific discipline "Bioorganic chemistry, chemistry of natural and physiologically active compounds".

04. 02. 2019

Opinion prepared by:

Assoc. Prof. Reneta Gevrenova, PhD