BULGARIAN ACADEMY OF SCIENCES

INSTITUTE OF ORGANIC CHEMISTRY WITH CENTRE OF PHYTOCHEMISTRY

R E VI E W

of

Prof. Vessela Deneva Kancheva, Dipl. Eng., PhD

Institute of Organic Chemistry with Centre of Phytochemistry - BAS

by competition: *occupying the academic position " Professor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.2. Chemical Sciences, in the specialty "Biochemical Chemistry, Chemistry of Natural and Physiologically Active Substances", at the Department of Biologically Active Substances, Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences(IOCCP-BAS), published in State Gazette, issue 43/31.05.2019.*

By Order No RD-09-187/26.07.2019 of the Director of the Institute of Organic Chemistry with Centre of Phytochemistry – Bulgarian Academy of Sciences, based on the decision of the Scientific Council of the Institute (protocol 18/11.07.2019) I was elected as a member of the scientific jury and for drawing up a review of the competition.

1. General description of the indicators for the materials presented

The submitted materials for the competition by Assoc. Prof. Dr. Petko Denev from IOCCP - BAS, which is the only candidate, are in accordance with the Rules for Scientific Development of the academic staff of IOCCP-BAS and meet the criteria for occupying the academic position of "Professor".

Of the 31 entries submitted for the competition:

- 22 are in the international journals with an impact factor (with a total IF = 52.39)

- distributed in quarters - 10 papers are with Q1, 4 - with Q2, and 8 - with Q4.

- without impact factor are 5 papers.

Some of the results of the research have been published in international journals with Q1 rank and high impact factor such as:

Industrial Crops and Products (IF 2015 - 3.449, Q1) - 2 papers,

Carbohydrate Polymers (IF 2017 - 5.158, Q1) - 2 papers,

Food Chemistry (IF 2018 - 5.399, Q1) - 3 papers,

Food and Chemical Toxicology (IF 2018 - 3.775, Q1) - 1 paper.

He presented also 3 chapters in collective monographs and presentations with reports in 75 international and national scientific forums.

Another useful feature is the presented utility model No. BG2031 / 01.04.2015 "Composition for synergistic enhancement of antioxidant activity of fruit and herbal extracts".

The reference for the fulfillment of the minimum national requirements for participation in a competition for the occupation of the academic position of "Professor" in the respective field of higher education shows that Assoc. Prof. Petko Denev meets the criteria for group A (Dissertation work for PhD - 50 points), and exceeds the criteria for:

group B (required 100points – 159points), group G (required 250points – 337pointst), group D (required 200points – 1062points) and group E (required 150points – 331points).

From the submitted documents and reports it is evident that in the announced competition Assoc. Prof. Petko Denev participates with a scientific asset, which fully meets the requirements and meets the criteria for occupying the academic position of "Professor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations of IOCCP - BAS.

2. Personal and professional details of the applicant

Assoc. Prof. Petko Denev, PhD from IOCCP-BAS was born on 10.06.1981. From 1995 to 1999 studied at the Vasil Drumev High School of Mathematics in Veliko Tarnovo. He graduated with a Bachelor's Degree from the University of Chemical Technology and Metalurgy (UCTM) - Sofia, majoring in Biotechnology and a Master's Degree at University of Food Technology (UFT) - Plovdiv majoring in Biotechnology in 2005. In 2011 Petko Denev received her PhD degree after successfully defending her dissertation on the study of ‘‘The antioxidant activity of anthocyanin - containing fruits and functional foods derived from them’’ under the guidance of Assoc. Prof. Maria Krachanova at UFT-Plovdiv. Since 2003 he has worked at the Bioactive Substances Laboratory at IOCCP-BAS consecutively as a chemist (2003 - 2007), assistant

(2007 - 2011), assistant professor (2011 - 2014) and associate professor since 2014.

Assoc. Prof. Petko Denev has continuously improved his qualification throughout his academic development. He holds specializations in:

- Belgium, KaHo Sint Lieven Technical University, 3 months, 2005.

- under the Socrates-Erasmus program; in Romania, Bucharest, Center for Biosensing and Biodynamics: Advanced Course in Biosensors, May 17 - June 2, 2006;

- in the Czech Republic, Czech Academy of Sciences, Institute of Biophysics, Laboratory for Pathophysiology of Free Radicals - 3 months (2006) and 2 months (2007); and

- in Slovenia, Maribor, University of Maribor, Laboratory for Separation Processes and Product Design, Project BG051PO001/3.3-05-0001 "Science and Business" under Operational Program "Human Resources Development" - "Selection of Postdoctoral Students and Young Scientists for one-month trainings in high-tech scientific complexes and infrastructures" 1 month, 2012.

Assoc. Prof. Petko Denev has a total of 88 publications with a total impact factor of over 100, which have been cited a total of 762 times and the H index 12 (by Scopus). He was the head of 2 research projects and the coordinator of IOCCP-BAS in 2 other projects funded by the National Science Fund. He is a participant in 10 projects funded by Bulgarian institutions, a scientific consultant in 2 projects, from the program for supporting young scientists at BAS, as well as a participant in 3 projects funded by Bulgarian private companies. For projects funded by EU Operational Programs, participates in 3, incl. 1 as a leader. For projects developed in international cooperation within the framework of inter-academic treaties and agreements - 3 as a leader.

Assoc. Prof. Petko Denev is a member of the Regional Union of NTS - Plovdiv, the Bulgarian Phytochemical Association and the Union of Chemists in Bulgaria. He is a long-time member of various expert committees: the COST (The European Cooperation in Science and Technology) program for the period 2019 - 2020; under Eurostars program co-financed by EUREKA and Horizon 2020: 2015, 2016, 2017; under Horizon 2020: TWINNING procedure: remote and central evaluation in Brussels, 06-10 July, 2015. He has been an expert-evaluator on projects at the Scientific Research Fund, 2016, 2017, 2018, 2019 years, as well as at the Science Foundation of UFT - Plovdiv, 2017, 2019 years and under the Scientific Research Fund of Plovdiv University "Paisii Hilendarski" - 2017 and 2019 years.

Assoc. Prof. Petko Denev participates in two organizing committees of international scientific forums:

- First International Conference on Bio-Antioxidants, 2017, Sofia, Bulgaria

- Third International Conference on Bio-Antioxidants, 2019, Nessebar, Bulgaria - as a coordinator of the Youth Bio-Antioxidant School.

Since 2014 he has successfully managed the Laboratory for Biologically Active Substances in Plovdiv.

3. Assessment of the applicant's research activity.

The research activity of Assoc. Prof. Petko Denev has been focused on scientific topics in an extremely important and relevant field. In recent years, special attention has been paid to socially significant and difficult to treat diseases of the people caused by the free radical processes in the body and the application of antioxidant therapy, which includes strengthening the antioxidant protection of the body against the harmful effects of free radicals. Age-related diseases are a social problem internationally and their prevention through natural products is a research area of particular interest. This has directed the scientific interest of Assoc. Prof. Denev to the study of the possibility of counteracting the risk factors of atherosclerosis that occurs in the process of aging. The results obtained by him can assist in finding and proposing effective preventive regimes against the initiation and development of diseases related to the harmful effects of free radicals.

4. ***Scientific Contributions***

The scientific and applied contributions from the works of Assoc. Prof. Petko Denev can be summarized in the following main topics, which are a priority for a number of national strategic documents such as the National Strategy for the Development of Research in the Republic of Bulgaria 2017-2030 "The Innovative Strategy for the Intelligent specialization":

1. *Investigation of the chemical composition and biological activity of medicinal and economically important plants*

In this topic, the scientific and applied work of Assoc. Prof. Denev is mainly focused on the fruits of Aronia melanocarpa, as it is among the richest sources of polyphenols and anthocyanins and is a suitable raw material for the production of functional foods. A large-scale study of the chemical composition of 23 aronia fruit samples from two consecutive harvests years (2016 and 2017) was conducted, which shows that there are significant

differences in their chemical composition, both in content and in composition of organic acids, sugars and phenolic compounds. Differences in the chemical composition of the raw chokoberries and variation of the technological parameters of the processing can lead to functional foods with different chemical composition, which is a prerequisite for different biological activity (paper 19). Quercetin and epicatechin have been observed to exhibit the strongest antioxidant activity among aronia polyphenols. The high amount of proanthocyanidins in fresh fruits is considered to be a major factor responsible for the high antioxidant activity. Aronia proanthocyanidins have also emerged as the most potent antimicrobial agents in the fetus (paper 24).

The technological trials conducted to synergistically increase the antioxidant activity of chokeberry and rosehip extract have led to a real practical application and a protected utility model. An animal model with healthy rats was used to study biological activity and the effect of chokeberry juice on various somatometric, lipid, and histological parameters in experimental animals was studied (Pub. 4). In the same animal model, animals supplemented with aronia were found to have reduced atherosclerotic lesions, confirming that aronia juice successfully retarded age-related vascular aging (paper 6) and may be recommended as a prophylactic agent for aging in good health. The effect of chokeberry juice on the learning ability and memory of adult rats as well as on their brain morphology was investigated. Aronia juice has been found to increase acetylcholinesterase activity in the hippocampus, which is a marker of improved functional activity of cholinergic neurons and an indicator of neuroprotective effect. These results indicate that chokeberry juice improves the ability of adult rats to learn tasks and improves their locomotor functions (paper 27).

The use of herbal extracts as co-pigments enables the development of functional chokeberry foods with improved sensory properties and biological effects due to the increased color stability and anthocyanins in them (paper 21).

Studies have been carried out and the phytochemical composition and biological activity of Bulgarian medicinal plants have been summarized for the antioxidant activity of nearly 80 Bulgarian fruits, vegetables and herbs and data for 11 Bulgarian mushrooms, 4 vegetables and 2 herbs have been added in a review article. The work was published as a chapter in a collective monograph (number 30).

Studies have been carried out on the application of medicinal plants in the development of

nutraceuticals and functional foods. This includes blackberry leaves (Rubus fruticosus), raspberries (Rubus idaeus), hawthorn (Crataegus monogyna) leaves, as well as the aerial parts of the lady's mantle (Alchemilla glabra) and meadowsweet (Filipendula ulmaria) aerial parts, which show very high in vitro antioxidant activity, measured by several methods - ORAC, HORAC, TRAP and lipid peroxidation inhibition. The meadowsweet saddle and blackberry leaves showed a very high antimicrobial effect (paper 1). It has also been found that M. officialis and H. perforatum extracts show the strongest antioxidant activity, which contributes most to the antioxidant activity of the antistress 1 and "antistress 2" products. For the first time, the antioxidant activity of various flavonoid glycosides isolated from herbaceous fruits (Sambicus ebulus) was determined (paper 2).

Recently, studies have been conducted on the kinetic parameters of co-pigmentation between strawberry anthocyanins and caffeic acids, which have shown that the co-pigmentation process is irreversible (paper 18).

For the first time, the chemical composition and biological activity of Chaenomeles maulei (Japanese quince, mountain lemon) - not very well known in Bulgaria - were studied. The juice has been found to be extremely rich in polyphenolic substances. The acidic taste of the juice and its low pH were due to the high content of organic acids (malic, citric, ascorbic and oxalic acids). Carbohydrates are represented by glucose,fructose, galactose, xylose, rhamnose and arabinose. The antioxidant activity was found to be very high and correlated with the high content of polyphenols. Based on these results, Prof. Denev correctly concludes that the fruits of C. maulei could be used in the food industry as a natural acidifying agent (paper 23).

As there is no comprehensive assessment of the genetic diversity of pepper (Capsum annuum) in terms of its antioxidant content, a study was conducted on the chemical composition and antioxidant activity of 63 genotypes in Balkan region (paper 26). The results obtained showed significant differences in the chemical composition between the different varieties and between the representatives in the groups. Prof. Denev correctly concludes that these variations in the chemical composition of fruits can be manipulated by plant propagation to create new varieties of pepper with improved nutritional value.

In another study, the basic parameters that influence the extraction process of polyphenolic compounds from tomatoes fruits were investigated and based on the kinetics of the extraction process; a theoretical model was proposed to describe the process (paper 2).

The results of the conducted studies with stevia (Stevia rebaudiana) lead to the conclusion that it is possible to develop genotypes of stevia with high content of stevio-glycosides on the path of individual selection and in the climatic conditions of Bulgaria (paper 9).
Egyptian quinoa seeds (Chenopodium quinoa) have been found to be good sources of essential nutrients such as minerals, essential amino acids and essential fatty acids (paper 13).

Direction 2 is aimed at the utilization of waste from the essential oil industry to obtain new products (publications 8,12,17,22,25).
I commend the results of these studies. Here, various polyphenolic and polysaccharide extracts from Rosa damascene, Calendula officinallis, Lavandula angustifolia and Melissa officinalis extracts have been obtained and characterized. Lavender and melissa waste are found to be rich in polyphenols (especially rosemary acid) and aromatic compounds and exhibit high antioxidant and antimicrobial activity. The total amount of dietary fiber increased threefold and the polyphenoyls four times in bread with 5% lavender and melissa waste added compared to the control sample and the shelf life of the bread increased to 96 hours with the additives. Of particular interest is the study on the synthesis of silver nanoparticles of Rosa damascena's waste extracts and their application for the electrochemical reading of carbon peroxide and vanillin. The electrodes developed show a high sensitivity and reproducible response for the quantification of hydrogen peroxide and vanillin.
Research in section 3 on the chemical composition and biological activity of pectic polysaccharides is also highly appreciated (publications 14, 15, 16, 20, chapter of monograph 29).
For the first time the polysaccharide composition in 11 European and in particular Bulgarian medicinal plants has been characterized. Long-term hot water extraction has been found to be a reliable way to obtain pectin-rich polysaccharide complexes with high in vitro complement-fixing activity and high ex vivo intestinal immunomodulatory effects. It was isolated for the first time from the colors of the linden a unique pectic polysaccharide with anti-inflammatory potential. The structure and immunomodulatory activity of the water extractable pectins frm lavender have been studied for the first time and lavender polysaccharides showing ex vivo immunostimulatory action on phagocytic leukocytes and intestinal immunomodulatory activity. Lavender pectins have been found to activate the inborn and acquired immune responses

through the blood. Also, the structure and immunomodulatory activity of water-extractable pectin polysaccharides from nettle leaves were studied for the first time. A large-scale study concluded that acidic hetero-polysaccharides of pectin type in Bulgarian medicinal plants exhibit a pronounced immunomodulatory activity with immunostimulatory and anti-inflammatory potential. The results obtained are of practical application and can serve as a basis for creating by-products of added value from cultivated Bulgarian herbs.

5. Assessment of the applicant's contribution

From the presented materials it is clear that to a great extent the results obtained and the formulated contributions are a personal merit of Assoc. Prof. Petko Denev. He is the first or co-author of 10 publications from all 31. The results are from work on 6 projects under his direction, funded by National and EU Operational Programs. He also directed large-scale chemical research composition and biological activity of some medicinal and economically important plants.

6.Critical notes and recommendations:

It is not clear what is the work of Assoc. Prof. Petko Denev in the last topic.

The educational activity of Assoc. Prof. Petko Denev expresses that he was the co-supervisor of 8 diploma works (probably for obtaining a master's degree) and a leader of 10 students in the framework of student internships. There is no doctoral guidance and student lectures. The latter is not required by the applicant, but I recommend that he she will continue to actively participate in the training of PhD students as a scientific adviser and with lectures at the BAS Training Center.

Conclusion:

From the submitted documents and reports it is evident that in the announced competition Assoc. Prof. Petko Denev participates with a scientific asset, which fully meets the requirements and meets the criteria for occupying the academic position of "Professor" according to the Act for the development of the Academic Staff in the Republic of Bulgaria and the Regulations of IOCCP - BAS.

Considering all of the above, my overall impression of the documents presented at the competition, as well as my personal excellent impression of the candidate, I am convinced that Associate Professor Petko Nedyalkov Denev fully meets the requirements of the Act for the development of the Academic Staff in the Republic of Bulgaria and the specific requirements of

IOCCP-BAS for occupation of the academic position of "Professor".

He is a scientist with a sufficient volume of scientific and applied scientific activity. On the basis of all this, I strongly recommend the honorable members of the scientific jury to vote positively for the award of the academic position of "Professor" in the scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" to Associate Professor Petko Denev from IOCCC - BAS.

05/09/2019 *Reviewer:*

(Prof. Dr. Vesela Kancheva)