PEER REVIEW

by Professor Diana Georgieva Ivanova, DSc in Biology, Professor in Biochemistry at Medical University "Prof. Dr. P. Stoyanov" - Varna

Regarding: a competition for acquiring the academic position "ASSOCIATE PROFESSOR" in the area of higher education ***4. Natural sciences, mathematics and informatics***, professional field ***4.3. Biological sciences*** and in the scientific specialty "***Biochemistry***", for the needs of the "Biochemistry, Molecular Medicine and Nutrigenomics" Department, Faculty of "Pharmacy" at the Medical University of Varna, according to a competition announced in the *State Gazette* No . 59/ July 26, 2022.

1. Brief information about the competition

Based on a decision of the Faculty Council of the Faculty of Pharmacy (Protocol No 59/July 26, 2022) and an order of the Rector of the Medical University of Varna (No P-109-375 dated September 26, 2022), I have been appointed as a member of the Scientific Jury, and according to Protocol No 1/ October 5, 2022 I have been assigned to prepare a peer review in relation to a procedure for tenure of the academic position of 'ASSOCIATE PROFESSOR' for the needs of the Department of Biochemistry, Molecular Medicine and Nutrigenomics at the Medical University of Varna, in the area of higher education ***4. Natural sciences, mathematics and informatics***, professional field ***4.3. Biological sciences*** and the scientific specialty of ***"Biochemistry"***.

The only candidate in the competition is Oskan Bahidinov Tasinov, Assistant-in-chief in the same department. The inspection of the documents indicates that they have been prepared correctly and meet the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its application.

2. Career profile of the applicant

Assistant-in-chief Oskan Bahidinov Tasinov was born on December 3, 1986 in Novi Pazar. After completing his high school education, he entered Sofia University "St. Kliment Ohridski". Kliment Ohridski", Sofia, from where in 2009 he acquired the Bachelor's Degree in Molecular Biology. In the period 2009-2011 he was a student at the Faculty of Biology of Plovdiv University "Paisii Hilendarski", acquiring the Master's degree in "Molecular Biology and Biotechnology" with a professional qualification of "Molecular Biotechnologist". Since January 1, 2016 he acquired a specialty at the Ministry of Health in "Biochemistry", a diploma from the Medical University of Varna. From 2012 to 2015 Oskan Tasinov was a PhD student and worked on a dissertation entitled

"*Study of antioxidant, anti-obesity and antidiabetic action of extracts of Sambucus ebulus L. fruit in vitro and in vivo*", which he defended at the Department of Biochemistry, Molecular Medicine and Nutrigenomics at the Faculty of Pharmacy of the Medical University of Varna. In 2015 he was awarded the educational and scientific degree "PhD" in the scientific specialty "*Biochemistry*". Oskan Tasinov's dissertation is a thorough and significant scientific study of the biological effects of dwarf elder fruits. The protective, antioxidant, anti-obesity and antidiabetic action of *Sambucus ebulus* fruit extracts in different experimental models *in vitro* and *in vivo* were analyzed. Phytochemical analysis of different infusions and extracts of *S. ebulus* fruits was performed and *in vitro* antioxidant activity, cytoprotective effect on 3T3-L1 preadipocytes in experimental models of induced oxidative stress and inflammation were determined, gene expression of proteins from intracellular antioxidant defense and cellular anti-inflammatory response were analyzed. In an interventional study, the influence of *S. ebulus* fruit tea on carbohydrate and lipid metabolism and immune response in healthy volunteers was examined. The dissertation of Oskan Tasinov for awarding the educational and scientific degree "PhD" headed by Prof. D. Ivanova, was awarded a diploma of the Union of Scientists in Bulgaria for Excellence in Theses for 2015. As a part of his training in the field, Oskan Tasinov conducted a short-term specialization at the Research Center of Molecular Medicine and Chronic Diseases (CIMUS) of the University of Santiago de Compostela, Spain with a scholarship of the Ministry of Education and Science under the "Science-Business" project, OP "Human Resources Development".

Oskan Tasinov started his work experience after graduating from Sofia University "Kl. Ohridski" in 2009, and since 2010 he has been appointed with a competition for Assistant Professor of Biochemistry at the Department of Biochemistry, Molecular Medicine and Nutrigenomics at the Medical University of Varna. After defending his dissertation in 2016, he appeared and won a competition for Chief Assistant at the same department where he currently works. He is a member of the Union of Scientists - Varna.

3. Teaching activities

O. Tasinov's teaching experience amounts to 12 years and 5 months. Assistant-in-chief Tasinov has good English language proficiency and conducts training in biochemistry and molecular biology both in Bulgarian and English for medical and dental students, participates in the teaching of pharmacy and technology transfer and innovations in pharmacy at MU-Varna. In addition to seminars and practical classes, O. Tasinov also gives separate lectures in the lecture courses in "Molecular biology" within the corresponding free elective courses and in the discipline "Research Technologies". Against an annual quota of 360 educational hours, Dr. Tasinov has a mean auditory

teaching loading during the recent 5 years which considerably surpasses the requirements for the position.

O. Tasinov is a co-author of 3 collections of assignments for dental medicine students in Bulgarian and in English and for kinesitherapy students published by teams from the Department.

4. Presented scientific production and scientific data

According to the presented list of scientific works, Dr. Tasinov participated in the competition with **13** articles in scientific editions abstracted and indexed in world-famous databases that provide to him **209** scores (indicator G-7) with minimum requirements of indicators G5-10 200 scores; a published monograph, submitted as a research work qualifying for an academic degree (indicator B3) entitled "Molecular mechanisms of immunomodulatory action – basis for the prophylactic and therapeutic potential of *Sambucus ebulus* L." – **100** scores and a dissertation work for the acquisition of the educational and scientific degree of 'PhD' – **50** scores (indicator A1). In five of the publications, Oskan Tasinov is the **first author**, in another five – the second, which demonstrates his active contribution to their elaboration and promotion. In the reference for the contributions, Oskan Tasinov points out two other full-text scientific publications in scientific journals that are not abstracted and indexed in world-famous databases of scientific information and are outside the minimum scientific-metric requirements for holding an academic position "Associate Professor". They do not bring additional points to the candidate, but are in the field of research on the biological activity of natural raw materials, which is why they are included in the review. Both the doctoral thesis and the publications of Dr. Tasinov are in the scientific field of the announced competition. The scientific publications of the candidate for the entire period of his career development amount to: 36 articles with a total volume of 306 pages, and 1 monograph with a volume of 167 pp.

The total number of citations in scientific journals, abstracted and indexed in world-famous databases (indicator D11), presented in Dr. Tasinov's Academic Transcript, is 25, which provide to him **50** points and meet the minimum requirements for the academic position "Associate Professor" in the professional field of the competition.

Six out of all the publications submitted are with a **total impact factor (IF)** of **20.094.**

The results of Dr. O. Tasinov's research have been presented to the scientific community at an impressive number of international (45) and national (12) congresses and symposia, one third of which are related to his dissertation. Although they are not reviewed, participations are taken into account when preparing the final opinion in the review.

During the years of his work as an Assistant Professor at the Department of Biochemistry, Molecular Medicine and Nutrigenomics Assistant-in-chief Tasinov takes an extremely active part in the work of the team on scientific projects – he is a member of the teams of 19 scientific projects.

Dr. Tasinov has active scientific profiles in:

- Google Scholar: https://scholar.google.com/citations?user=5Qr6QbMAAAAJ&hl=en&oi=ao
- Orcid: https://orcid.org/0000-0001-7268-3937
- Scopus: https://www.scopus.com/authid/detail.uri?authorId=56009691000
- ResearchGate: https://www.researchgate.net/profile/Oskan-Tasinov

5. Assessment of the research activity and scientific contributions

The scientific research of Dr. Oskan Tasinov is in two main thematic areas in the field of medico-biological sciences, with a view to maintaining human health and early diagnosis of diseases:

1. Food and nutrition

1.1. Evaluation of biological effects of medicinal plants.

1.2. Study of the biological effects of water and food additives containing micro- and macro-elements and their salts.

2. Oncology – study of predictive and prognostic-diagnostic biomarkers in colorectal cancer.

The contributions of Dr. Tasinov's scientific publications are mainly related to the assessment of the biological effects and healing potential of the dwarf elder plant, *Sambucus ebulus* L. I accept all contributions in their order according to significance and scientificmetric criteria:

1. Food and nutrition

1.1. Evaluation of biological effects of medicinal plants: A1; C3; G7-01; G7-02; G7-04 to G7-06; G7-11; Suppl. №1 - 2.

The dissertation thesis for acquiring the Educational and Scientific Degree "PhD", as well as a significant number of the publications of O. Tasinov (G7-01; G7-02; G7-04 to G7-06; G7-11); are devoted to analyses of the biological effects and healing potential of the fruits of the dwarf elder plant. Upon determining the phytochemical composition of different types of infusion and extracts from fruits their antioxidant, anti-inflammatory and immunomodeling capacity in models of induced inflammation or oxidative stress were established in cell cultures. A rich set of models was applied, on different types of cells (3T3- L1 preadipocytes and J774A.1 macrophages), the effect of fruits on transcription of enzymes from antioxidant protection, of factors involved in inflammation, NF-

κB, enzymes related to phagocytosis, etc. was studied. Interventional studies involving healthy volunteers confirmed the antioxidant potential of the fruits and at the molecular level. By monitoring changes in the expression of proinflammatory cytokines, adhesion factors and genes associated with the insulin signaling pathway, it was established that the tea intake favorably affected lipid metabolism in humans and had an anti-obesity potential, antiatherogenic and antidiabetic activities.

Dr. Tasinov's monographic work presents a focus on the research of the immunomodulatory action of *S. ebulus* as a basis for the prophylactic and therapeutic potential of the herb. A comparative analysis examines the nature and mechanism of action of chemical and synthetic immunomodulators, on one hand, and, on the other, immunomodulators of plant origin. Major targeted by the plant immunomodulators signaling and metabolic pathways are discussed in relation to the anti-inflammatory response. An overview of the application of *S. ebulus* in folk medicine on the Balkans, Asia Minor and the Middle East is made. Modern scientific studies on phytochemical composition, data on antioxidant activity, anti-inflammatory action, anticancer action, antibacterial, antiviral, antifungal and antiparasitic action of isolates and products obtained from different parts of the dwarf elder are presented in detail. Data are also presented about other biological effects of *S. ebulus* (antidepressant, antiemetic, neuroprotective, antihemolytic and in reproductive disorders). The monograph also includes own results as examples of modern scientific studies in the field and it is a contribution to the discovery of the molecular mechanisms of action of this medicinal plant with ubiquitous distribution and use in the Balkans. Dr. Tasinov also took part in a study that demonstrated for the first time the anti-inflammatory and antioxidant potential of the whip plant (*Agrimonia eupatoria* L.) after the consumption of agrimony tea by healthy volunteers with a normal body mass index. In an *ex vivo* model stimulated with bacterial LPS peripheral blood mononuclear cells (PBMC), a decrease in LPS-stimulated cytokine transcription was demonstrated and anti-inflammatory potential of *A. eupatoria* in humans was established. In these full-text publications beyond the minimum scientificometric requirements for holding of educational and scientific degree "Associate Professor" the previously created model of *ex vivo* stimulation with LPS of PBMC from whole human blood (suppl. **1**) is described. The second publication (suppl. **2**) presents first data on the antiinflammatory effect of a black mulberry heartwood (*Morus nigra* L.) in mouse macrofage cells.

1.2. ***Study of the biological effects of water and food additives containing micro- and macro-elements and their salts.:*** G7-03; G7-07; G7-10; D7-12.

Assistant-in-chief Oskan Tasinov participated in two studies that explored the biological action of water: waters from wetlands in the territory of Bulgaria (**G7-03**) and sulfur-containing

mineral waters from the territory of the town of Varna (**G7-07**). For the first time the cytotoxic effect of waters from natural reservoirs in Bulgaria with proven development of toxigenic cyanoprokaryotes was demonstrated in Hs27 cell line of human skin cells. On the other hand, the first sulfur-containing mineral water intervention with healthy volunteers, was carried out demonstrating that sulfur-containing water intake improved redox status in humans, increasing plasma levels of total glutathione and total thiols and stimulating the transcription of sICAM-1 and the antioxidant enzyme GCL, with a potential role of the sulfur-containing compounds in water.

An investigation of the molecular mechanisms of action of Ferrum phosphoricum (FP) D12 (**G7-10**) on cell proliferation and transcription of genes associated with iron metabolism, antioxidant defense and inflammation has shown for the first time a significant stimulation of the transcription of ferritin and other proteins involved in iron metabolism, of the antioxidant enzyme GPx-1 and the cytokine IL-1β, using J774A.1 macrophages.

A study on the biological effects of melatonin (**G7-12**) found that its pharmacological application exerted a prominent antioxidant effect on the expression and activity of antioxidant enzymes in a model of burn-induced oxidative stress in rats: it significantly enhanced the burn-induced activity of Cu/Zn SOD and lowered the transcription of the antioxidant enzymes glutathione peroxidase, glutathione reductase and catalase.

2. Oncology – study of predictive and prognostic-diagnostic biomarkers in colorectal cancer: G7-08; D7-13.

The results of the candidate from studies on small RNAs as prognostic markers in patients with metastatic colorectal cancer (**G7-08**) are of interest from a theoretical and practical point of view. Overexpression of circulating miR-618 was detected in the serum of these patients, and significantly longer mean overall survival was found for patients with high and intermediate miR-618 expression, in contrast to those with low expression. Additionally, the SNP genotype of miR-618 is a determinant of the risk of developing colon cancer. The review publication provides an in-depth review of circular RNAs (circRNAs) with their unique characteristics, such as potential prognostic and diagnostic biomarkers in colorectal cancer.

A contribution to clinical and laboratory practice is the published advanced combined method for the extraction of RNA from fixed in formalin and embedded in paraffin (FFPE) biopsy tissues, application of which increases the sensitivity and quality of the subsequent qPCR analysis (**G7-13**).

The candidate's contributions to the above areas demonstrate professional competence in the field of medico-biological research, including in terms of transferring scientific results in an applied context. A completely new look at the use and health effects of *Sambucus ebulus*, the many new

data on the phytochemical composition and biological effects of the plant, represent a significant scientific contribution, which is particularly impressive. The scientific developments of high scientific value and the active participation of O. Tasinov in the scientific projects of the department characterize him as an ambitious, inquisitive, consistent and competent young scientist who applies a complex approach and critical thinking in solving current scientific problems. I hope that the rapid professional development and experience gained will allow him to timely orient himself in a major area of scientific interest, which he will continue to actively develop in the future by creating his own group of followers.

6. Conclusion

Assistant-in-chief Oskan Tasinov is a very well trained specialist and lecturer in biochemistry at the Department of Biochemistry, Molecular Biology and Nutrigenomics at the Medical University – Varna. The analysis of the scientific production, teaching experience and experience gained as a participant in scientific teams gives me reason to assume that Dr. Tasinov meets all the requirements of the Law for development of the academic staff in the Republic of Bulgaria, of the Regulations for its application, and of the Regulations for development of the academic staff in the Medical University of Varna for tenure of the academic position of 'associate professor'. I strongly recommend to the esteemed jury to award to Dr. Oskan Bahidinov Tasinov the academic title "Associate Professor" in the area of higher education ***4. Natural sciences, mathematics and informatics***, professional foeld ***4.3. Biological sciences*** and scientific specialty "***Biochemistry***", for the needs of the "Biochemistry, Molecular Medicine and Nutrigenomics" Department, Faculty of "Pharmacy" at the Medical University of Varna.

November 30, 2022 г.

Reviewer:

/Prof. Diana Ivanova, DSc/