

## PEER REVIEW

by Professor Diana Georgieva Ivanova, DSc in Biology, Professor in Biochemistry,  
at the Medical University 'Prof. Paraskev Stoyanov' of Varna

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

### 1. Brief information about the competition

Based on the decision of the Faculty Council of the Faculty of Pharmacy (Protocol No. 41/19.09.2022) and the order of the Rector of the Medical University - Varna (No. P-109-359 of 21.09.2022), I have been elected as a member of the Scientific Jury, and by Protocol No. 1/05.10.2022, I was assigned to prepare a peer review in relation to a procedure for tenure of the academic position of "PROFESSOR" for the needs of the Department of "Biochemistry, Molecular Medicine and Nutrigenomics" at the University of Varna, in the area of higher education 4. *Natural sciences, mathematics and informatics*, professional field 4.3. *Biological sciences* and scientific specialty of "*Biochemistry*".

The only candidate in the competition is Yoana Dimitrova Kiselova-Kaneva, Associate Professor in the same department. The inspection of the documents indicates that they have been prepared precisely and fully meet the requirements of the Law for development of the academic staff in the Republic of Bulgaria and the Regulations for its implementation..

### 2. Career profile of the applicant

Assoc. Prof. Yoana Dimitrova Kiselova-Kaneva was born on January 03, 1976 in Gabrovo. She completed her higher education in 1999 at the Plovdiv University "Paisiy Hilendarski" and obtained a Master's degree in "Biology" with a specialization in "Genetics and Cell Biology". In the period 2004-2009, she performed a specialization to the Ministry of Health at the Medical University "Prof. Dr. P. Stoyanov", Varna, certificate of recognized specialty in "Biochemistry" No. 2742 dated 20.02.2009.

In the period 2004–2006, she was a full-time doctoral student at the Department of Preclinical and Clinical Pharmacology, Chemistry and Biochemistry of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, and since 2006 she has been transferred as an independent PhD student to the newly established Department of Biochemistry, Molecular Medicine and Nutrigenomics. In 2012, she defended her dissertation thesis and was awarded the educational and scientific degree of 'PhD' in the scientific speciality of "Biochemistry" on the topic "Study of the antioxidant activity of Bulgarian medicinal plants".

In her PhD thesis, Assoc. Prof. Yoana Kiselova investigated the *in vitro* antioxidant activity and polyphenol content of extracts and fractions of 54 medicinal plants that used in Bulgarian folk medicine. Based on the analysis of the obtained results, the agrimony plant (*Agrimonia eupatoria* L.) was selected, and its extract was studied in detail in experiments with cell cultures and



experimental animal models. In a model of induced oxidative stress in preadipocyte (3T3-L1) and macrophage (J774A.1) cell cultures, the antioxidant potential of *A. eupatoria* was demonstrated by examining the effects on cell viability and the expression of genes related to antioxidant defense and inflammation. In an experimental model with fructose loading to induce metabolic disorders in rats, a protective effect of the intake of an aqueous extract of *A. eupatoria* was found on the oxidative status in serum and tissue homogenates, and also on gene expression in adipose tissue.

After her graduation in 1999, Y. Kiselova joined the Institute of Genetics "Acad. Doncho Kostov" at the Bulgarian Academy of Sciences, Sofia, where from 2000 to 2004 incl. hold the position of research assistant III degree. Since 2006, she was appointed "Assistant Professor" in biochemistry to the Department of Preclinical and Clinical Pharmacology, Chemistry and Biochemistry at the Medical University "Prof. Dr. Paraskev Stoyanov", city of Varna. From 2009 to 2011 she was a Senior Assistant, and from 2011 to 2013 - an Assistant-in-chief at the newly established Department of Biochemistry, Molecular Medicine and Nutrigenomics. In 2013, she qualified as an "Associate Professor" at the same department, and from 2016 until now she is the head of the Department of Biochemistry, Molecular Medicine and Nutrigenomics. Since 2017, she has been appointed as the head of the "Nutrition and Quality of Life" Department at the newly established Scientific Research Institute at the Medical University "Prof. Dr. Paraskev Stoyanov", Varna, while at the same time she is the head of the scientific group "Nutrigenomics and personalized nutrition" within this department.

As part of her professional training in the field, Assoc. Prof. Kiselova participated in nine different training courses, for which she has presented certificates. She is a member of the Union of Scientists in Bulgaria.

### **3. Teaching activity**

Assoc. prof. Kiselova has over 20 years of teaching experience. She is fluent in English and conducts training in biochemistry and molecular biology in Bulgarian and English for students of medicine and dentistry, participates in the teaching of pharmacy and transfer of technologies and innovations in pharmacy programmes and in the teaching of biochemistry and pathobiochemistry at the Medical College of Medical University - Varna. Assoc. Prof. Kiselova gives lectures, seminars and exercises in the disciplines "Biochemistry", "Research Technologies", "Molecular Biology in Medicine", "Molecular Biology in Pharmacy", "Biological role, mechanism of action and pharmacological application of macronutrients and their salts", single lectures in "Pharmacognosy".

Against an annual quota of 126 educational hours, Assoc. Prof. Y. Kiselova has a mean auditory teaching loading during the recent 5 years which considerably surpasses the requirements, according to the report provided to me by the Academic Activities Directorate, and amounts to 155 hours. She leads and periodically updates the biochemistry lecture courses for students of dental medicine, and has also independently developed the elective course "Biological role, mechanism of action and pharmacological application of macronutrients and their salts".

Assoc. prof. Kiselova is a co-author in a total of 4 university textbooks published by teams from the department - collections of questions and tasks for medical students, in Bulgarian and English, for dental medicine students, in English, and for pharmacy students, with a total volume of 574 pages.



She is the supervisor of two successfully defended doctoral students: Neshe Ferahova Nazifova-Tasinova, with the topic of the dissertation "Identification of biomarkers for the assessment of phenotypic plasticity with application in nutrition science" (2015) and Miglena Nikolaeva Todorova, with the topic of the dissertation thesis "Obtaining anthocyanin rich extract from the fruits of elderberry (*Sambucus ebulus*) and characterizing its biological activity with a view to its use as a raw material in the production of food and medicinal products" (2019), which provides her with 100 scores in indicator E13.

#### **4. Scientific production submitted and scientometric data**

In the current competition for a "Professor", Assoc. Prof. Yoana Kiselova-Kaneva participated with 27 scientific works, arranged according to a model, proving the fulfillment of the minimum requirements for occupying the academic position of "Professor":

A1: Doctoral thesis for the acquisition of the educational and scientific degree of 'PhD' – 50 points;

C4: Scientific publications in journals that are abstracted and indexed in world-renowned databases of scientific information, equivalent to a habilitation thesis – 6 items, equal to 101 scores where 100 scores are required in indicator C;

G7: Scientific publications in journals that are abstracted and indexed in n world-eminent data-bases of scientific information – 18 items, that provide to her 287 scores and G8: 2 published book chapters in English – 30 scores, in summary 317 scores, where a minimum of 200 scores are required in indicator G.

The research papers in which the candidate is a co-author are of a complex nature and some are performed by larger teams. Nevertheless, she is the first, second or lead last author in 1/3 of them, indicating her active contribution to their development and promotion. In the academic reference, Assoc. Prof. Kiselova also indicates four other full-text scientific publications in scientific journals, which are not referenced and indexed in world-eminent databases with scientific information and are outside the minimum scientometric requirements for occupying the academic position "Professor". They do not bring additional scores to the candidate, but they are in the same field of research, with a significant contribution from a methodological point of view, which is why they are being reviewed. Both the PhD thesis and the publications of Assoc. Prof. Kiselova are in the scientific field of the announced competition. The candidate's scientific publications for the period from 2001 to the present amount to: 68 articles and 1 monograph, participation in over 60 scientific forums and numerous (over 20) scientific projects.

The entire scientific production of the candidate is taken into account when preparing the final opinion in the review.

The total number of citations in scientific publications, abstracted and indexed in world-eminent databases (indicator D11), presented in the Academic Reference of Assoc. Prof. Kiselova, is 86, which provides 172 scores and significantly exceeds the minimum requirements for "Professor" in the professional field of the contest.

Of all publications submitted for participation in the competition, 14 have a total impact factor (IF) of 45,838.

Assoc. Prof. Kiselova actively works as a researcher and expert in 7 national (70 scores under E14) and one international (20 scores under E15) scientific projects, and in recent years she is the author



and leader of two other projects (40 scores in total according to indicator E16). Thus, in indicators from group E (supervision of successfully defended doctoral students' theses, participation in and management of projects, and attracted funds for projects led by the candidate, 24 scores, as well as participation in collections of study materials for students (13.83 scores), Assoc. Prof. Kiselova is granted in total 267, 83 scores, with minimum requirements of the indicators for 100 scores.

The management of an established scientific group working on modern problems of science and technology (Assoc. Prof. Kiselova is the head of the "Nutrition and Quality of Life" department at Research Institute within the Medical University of Varna), as well as the management of the scientific group "Nutrigenomics and personalized nutrition" to this direction, I refer deservedly to the development, appearance and recognition of the candidate as an established scientist in the field of nutrigenomics.

Assoc. Prof. Kiselova has active scientific profiles in:

Google Scholar: <https://scholar.google.com/citations?hl=en&user=Bp6X84gAAAAJ>

Orcid: <https://orcid.org/0000-0001-9692-6227>

ResearchGate: <https://www.researchgate.net/profile/Yoana-Kiselova-Kaneva>

## 5. Evaluation of the scientific production and scientific contributions

The publications presented by Assoc. Prof. Kiselova-Kaneva are thematically grouped into the following three main thematic directions in the field of medico-biological sciences:

### 1. *Biological activity, composition, metabolism and safety of natural raw materials, food additives and synthetic molecules (B4.1, B4.2, B4.3, B4.4, B4.5, B4.6, G7.1, G7.2, G7.3, G8.1, G8.2)*

#### 1.1. Study of the fruits of the medicinal dwarf elder plant (*Sambucus ebulus* L.) with a view to their use as raw material in the production of food and therapeutic agents

A significant number of the publications submitted for review in the competition by Assoc. Prof. Kiselova are devoted to the study of fruits of the medicinal plant dwarf elder (*Sambucus ebulus* L.) with a view to their use as raw material in the production of food and therapeutic agents. The scientific works in this direction (B4.1, B4.2, B4.3, B4.4, B4.5 and B4.6) are presented as the equivalent of a monographic/habilitation work. Inspired by the long history of the use of elderberries in the traditional medicine of the Balkans and the preparation of home medicinal preparations and foods from the fruits by the population in Bulgaria, and at the same time poorly studied chemical content and biological activity, the department is developing a number of scientific projects on the subject, initiated by Assoc. Prof. Kiselova. The scientific evidence for the potential anti-inflammatory and/or immunomodulatory properties of elderberries is sought. Detailed phytochemical characterization of *S. ebulus* fruit extracts (B4.1, B4.2, B4.3, B4.4) was performed. The first scientific reports were made about the following contained in the fruits: 3 types of anthocyanins - predominantly cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside and cyanidin-3-O-xyloside (B4.2); 9 types of free and conjugated hydroxycinnamic acids; the flavonols quercetin and kaempferol (B4.3); isorhamnetin (7-hydroxyflavonol) (B4.4); 12 types of flavonol glucosides



(B4.1, B4.2, B4.4); trans-resveratrol (B4.1, B4.2) and aglycone (B4.4); 3 types of essential amino acids: isoleucine, phenylalanine and lysine (B4.2); 7 types of organic acids (B4.2); 17 types of sugar alcohols and their derivatives, of which the highest concentration is sorbitol (B4.2); 12 types of sugar acids and their derivatives (B4.2); 23 types of mono-, di- and tri-saccharides and their derivatives (B4.2); 2 types of fatty acid esters<sup>1</sup> (B4.2);  $\beta$ -sitosterol.

Confirmational data were obtained for: cyanidin-3-O-glucoside (anthocyanin) (B4.2); 3-O-caffeoylquinic acid (chlorogenic acid) (hydroxycinnamic acid) - quantitatively determined for the first time (B4.1, B4.2); catechin and epicatechin (proanthocyanidin monomers), as well as proanthocyanidin mono-, di- and trimers (B4.1, B4.2); 3 essential amino acids: valine, leucine and threonine (B4.2); fumarate, malate and succinate (B4.2); glucose and sucrose (B4.2). According to the available literature data, extracts of elderberries have higher content/g dry weight of most of the studied compounds, compared to *S. nigra* or other small fruits and are found to be suitable as ingredients in foods, nutritional supplements, medicinal remedies etc. Given the fact that the concentration found in tea (6.54 mg/g DW) and aqueous extract (cold extract) (5.19 mg/g DW) of *trans*-resveratrol (glycoside form) is many times higher than the reported content of it in the skin of grapes (mean 0.169 mg/g DW), the elderberries are of interest in terms of the industrial extraction of this compound or resveratrol-rich extracts.

Upon establishing the phytochemical composition of different types of fruit extracts and infusions, their antioxidant, anti-inflammatory and immunomodulating capacities were established in models of induced inflammation or oxidative stress in cell cultures. A wide range of models was applied, on different types of cells (3T3-L1 preadipocytes and macrophages (J774A.1), the effect of fruits on the transcription of enzymes of antioxidant defense, factors involved in inflammation, NF- $\kappa$ B, enzymes, related to phagocytosis, etc. *S. ebulus* fruit extracts were found. The expression of genes related to inflammatory response, antioxidant defense and endoplasmic reticulum stress (B4.2, B4.4, B4.5 and B4.6) is affected. Applied *in vitro* in cell cultures, it was found that elderberry extracts had different effects on the expression of genes related to the inflammatory response, antioxidant defense and endoplasmic reticulum stress, depending on whether they were administered in "unstimulated" cells or on cells under oxidative/proinflammatory stimuli - "stimulated cells". (B4.2, B4.4, B4.5 and B4.6). For the first time, a cytoprotective effect of extracts from fruits of *S. ebulus* in cell cultures was established (B4.4, B4.5). The results about the biological activity of the fruits support its use in folk medicine, demonstrate an immunomodulatory effect and a potential to prevent cell death, oxidative stress and to control inflammatory processes.

#### 1.2. Investigation of the metabolic effects of sulphur-containing mineral waters from the Varna Basin (G7.1)

The mineral waters of the Varna basin, which are available for public use, are believed to have a beneficial effect on the gastrointestinal and excretory systems. However, there is a lack of systematic scientific research on their composition and physiological activity. In a project study with the participation of Assoc. Prof. Kiselova, the main physicochemical characteristics of sulfur-containing mineral waters from two springs in the city of Varna were determined. An interventional study found a beneficial effect on antioxidant capacity after eight weeks of mineral water intake in healthy volunteers: an increase in plasma levels of total thiols, glutathione and in the expression (mRNA) of GCL and sICAM-1 in peripheral mononuclear cells was found.



### 1.3. Toxicity of cyanotoxins and waters contaminated with cyanotoxins (G7.2, G7.3, G8.1)

Data were obtained on species diversity and cytotoxicity of blooming waters from industrial and recreational water bodies in *in vitro* experiments on skin fibroblasts (HS-27). Cytotoxicity corresponded to toxigenic species of cyanoprokaryotes and the presence of cyanotoxins in these waters. New data on various aspects of toxicity/safety of cylindrospermopsin toxin are reported: on the ATPase and diamine oxidase activities of purified enzyme and in isolated Wistar rat liver cells; on the frequency of cardiac contraction in a prepared frog heart; the inotropic effect of obestatin (G7.2). Its toxicity has been demonstrated in cell culture (human intestinal HIEC-6 cells) (G7.3). In a review article, literature data on the toxicity and carcinogenicity of cyanotoxins are summarized and the possibilities for the use of some as molecules with antitumor and other therapeutic actions are discussed. (G8.1).

### 1.4. Gastroprotective effects of melatonin (Г7.4)

New data on the effect of melatonin on the expression of antioxidant defense genes in the gastric mucosa in a Wistar rat burn trauma model are reported: twofold increase in the amount of Cu/Zn superoxide dismutase in the gastric mucosa as compared to the untreated burn control; reduced stimulatory effect of burn trauma on the transcription (mRNA) of enzymes involved in antioxidant defense - GPx, Cat and Gsr.

### 1.5. Biological effects of Ferrum phosphoricum (FP) (Г7.5)

A study of the molecular mechanisms of action of Ferrum phosphoricum (FP) D12 on cell proliferation and the transcription of genes related to iron metabolism, antioxidant defense and inflammation shows for the first time a significant stimulation of the transcription of ferritin and other proteins of iron metabolism, of the antioxidant enzyme GPx-1 and cytokine IL-1 $\beta$  in J774A.1 macrophages

### 1.6. Iron content in nutritional supplements (Г7.7)

A study of the iron content in 29 types of food supplements from the commercial network found that it did not correspond to label indications: in 52% of the analyzed samples it exceeded indicated on the label, and in the remaining 48% it was lower. The established deviations are within +56% and -83%, and 14% of the preparations do not meet the European standards. In some formulations, the dosage recommended by the manufacturer differs from the recommended daily dose.

### 1.7. A newly synthesized organophosphorus compound - physicochemical analysis, metabolism and biological activity (Г7.6)

In collaboration with a team from Shumen University "Bishop Konstantin Preslavski", an organophosphorus compound was newly synthesized by the team (5-Ethyl-5-methyl-4-bromo-2-N-butylamido-2,5-dihydro-1,2-oxaphosphol-2-oxide, Br-oxph-1) in order to develop new biologically active compounds, including drugs. Physicochemical characterization of Br-oxph-1 indicates that the ability to cross membranes is a prerequisite for expected effects on cellular metabolism. For the first time, the metabolism of Br-oxph-1 and its possible molecular interactions were modeled *in silico*. 37 probable products of hepatic metabolism of the compound and some molecular interactions of the corresponding metabolites were predicted. A proliferative effect of low concentrations of Br-oxph-1 was found in J774A.1 macrophage cell culture, stronger toxicity in



tumor cells, compared to normal J774A.1 macrophages. Low, non-toxic concentrations of Br-oxph-1 stimulate the expression of GCL and GPx enzymes associated with antioxidant defense.

#### 1.8. Antioxidant activity and synergistic effects of taxifolin and fucoidan (Г7.8)

The mechanisms of interactions between synthetic or natural antioxidants as active ingredients or excipients in multicomponent mixtures is of interest for the standardization and optimization of pharmaceutical formulations. Mixtures of taxifolin (dihydroquercetin) and fucoidan were investigated with a view to developing preparations for the prevention and treatment of conditions caused by oxidative damage. An *in vitro* antioxidant synergistic effect of taxifolin and fucoidan was established. The most pronounced antioxidant properties is the taxifolin/fucoidan mixture in a ratio of 1:3, followed by taxifolin/fucoidan 1:1 and taxifolin/fucoidan = 3:1. On its own, taxifolin has higher antioxidant activity than fucoidan - it produces a 50% effect at almost three times lower concentration than fucoidan and reaches its highest antioxidant activity at a lower concentration than fucoidan.

#### 1.9. Antioxidant activity of bexarotene derivatives (G7.9)

Bexarotene is a third-generation synthetic retinoid used in the treatment of cutaneous T-cell lymphoma. Antioxidant activity of five hydrazone derivatives of bexarotene, the one with the highest activity according to an electrochemical method, was established. DPPH neutralization and ABTS radical decolorization methods did not establish antioxidant activity of the studied compounds.

#### 1.10. Total polyphenolic content and antioxidant activity of beverages from the commercial network in Bulgaria (G7.10)

In a comparative analysis, data were obtained on the antioxidant activity and total polyphenol content in alcoholic and non-alcoholic fruit drinks on the Bulgarian market. Of the red wines with the highest content of polyphenols, Merlot wines are distinguished, and Syrah is the lowest; the richest in anthocyanins is Malbec, and with the highest antioxidant activity - chokeberry wine. Aronia juice and compote had the highest polyphenol content among the soft drinks tested.

#### 1.11. Antidiabetic potential of medicinal plants (G8.2)

A detailed literature review was discusses 42 species of medicinal plants and plants from the Bulgarian flora regarding possible medicinal use in type 2 diabetes patients; mechanisms of hypoglycemic and hypolipidemic action for some of the plants, target molecules and processes are summarised.

### **2. *Molecular markers in search of new diagnostic and prognostic approaches (G7.4, G7.5, G7.6, G7.7, G7.8, G7.8, G7.10, G7, G7.11, G7.12, G7.13, G7.14, G7.15, G7.16, G7.17)***

#### 2.1. Plasma levels and expression of matrix Gla-protein and vitamin D status in patients with cardiovascular disease (G.11, G7.12, G7.13).

Plasma levels and expressions of matrix Gla-protein and vitamin D status are investigated as potential molecular markers in patients with cardiovascular disease (CVD). Data on plasma levels



of uncarboxylated MGP (ucMGP) (G7.11) in patients as compared to a control group of clinically healthy volunteers at risk of CVD indicate almost twice increase for patients with heart failure. As the calcium score increases, so does the plasma concentration of ucMGP. An inverse correlation was found between the plasma concentration of ucMGP and the Castelli risk index (TC/HDL-C), both in the entire studied group of patients and in the separate groups of controls and those with atrial fibrillation. An association between low levels of vitamin K and high levels of ucMGP was shown, higher levels of ucMGP in women, lower in obesity and in higher body mass index and in arterial hypertension.

A study of MGP expression levels in peripheral mononuclear cells (PBMCs) from patients with CVD (G7.12) found an association between high expression and high calcium score. MGP mRNA levels tended to be lower in all subgroups of patients with etiologic CVD risk factors, but were significantly (half) lower in CVD patients with higher total and LDL cholesterol levels. They are also lower in individuals with higher plasma levels of ucMGP.

Data on vitamin D status and CYP27B1 (1- $\alpha$ -hydroxylase) expression levels in PBMCs of CVD patients (G7.13) indicate that serum 25OHD levels in CVD patients are lower than in clinically healthy controls at risk of CVD but that decrease with increasing severity of CVD pathology. Circulating vitamin D (25OHD), and also 1- $\alpha$ -hydroxylase (CYP27B1), a regulatory enzyme of vitamin D synthesis, were at lower levels in the group of patients with a higher calcium score, with a significant positive correlation between serum levels of 25OHD and 1- $\alpha$ -hydroxylase expression in PBMC and they were significantly lower in CVD patients on statin therapy.

## 2.2. Molecular markers in children's saliva (G7.15, G7.16)

Interest in the application of minimally invasive diagnostic procedures is increasing, especially when it comes to diagnostics in children. Saliva is a biological substrate that includes more than 3,000 types of mRNA that could serve as biomarkers regarding various health disorders. The anatomophysiological features of the salivary glands are a prerequisite for a real molecular exchange of biomarkers from the blood to the acini, and from there to the saliva. IL-6, MMP-8 (matrix metalloproteinase-8) and GSS (glutathione synthetase) mRNA levels in saliva of children with pyelonephritis were investigated to evaluate their applicability as diagnostic markers. They settle down higher levels for IL-6 compared to saliva samples from healthy children, correlation between IL-6 mRNA levels and GSS and blood leukocyte counts in patients with pyelonephritis, increased salivary levels of dextran and IgA in children with gingivitis compared to a control group of healthy children.

## 2.3. Markers of metabolic adaptation (G7.14)

An international study tracked the effect of reduced caloric intake on phenotypic plasticity. In an interventional trial with calorie restriction in overweight people, changes in classical biochemical parameters, changes in the way individuals respond to metabolic stress (oral glucose tolerance test (OGTT)) were monitored, and panels of metabolic biomarkers were identified. Response in the OGTT postprandial changes of selected fatty acids and complex lipids, bile acids, amino acids, acylcarnitines and sugars such as mannose were found to reveal significant differences in the responses observed in individuals differing in postprandial glucose levels, glucose clearance and fasting plasma concentrations of more than 20 known biomarkers of insulin resistance and diabetes.



Analysis of the corresponding metabolic profile in a fasting blood sample allows early identification of pre-diabetic subjects at risk of insulin resistance without the need to undergo of the OGTT.

#### 2.4. Sulfated glycosaminoglycan secretion levels in obese type 2 diabetic patients on metformin therapy (G7.17)

In the pathogenesis of diabetes and its complications, e.g. diabetic nephropathy, a significant role is played by disturbances in the metabolism of components of the extracellular matrix, including proteoglycan aggregates. Changes in the metabolism of these macromolecules can lead to disturbances in the structure and function of the extracellular matrix and contribute to various complications in type 2 diabetes (T2D). The influence of metformin used to treat patients with T2DM on the type and amount of glycosaminoglycans excreted in the urine was evaluated. Data were obtained on the urinary excretion spectrum of total sulfated glycosaminoglycans (GAG) and their specific types: chondroitin sulfate/dermatan sulfate (CS/DS) and heparan sulfate (HS) in obese T2D patients treated with metformin monotherapy for a period for six months. Significantly higher total GAG, CS/DS and HS levels were found in untreated diabetic patients compared to healthy subjects. Their levels significantly decreased after six months of metformin therapy compared to those before treatment.

#### **3. *Development and adaptation of new preparatory and analytical methods (B4.3, F7.18)***

Contributing to clinical and laboratory practice is the published improved combined method for RNA extraction from formalin-fixed, paraffin-embedded (FFPE) biopsy tissue, the application of which increases the sensitivity and quality of subsequent qPCR analysis (G7.18).

In connection with the performance of phytochemical analyzes of medicinal plant extracts at the Department of Biochemistry, Molecular Medicine and Nutrigenomics, an LS-MS method was developed for the measurement of some phenolic compounds in plant extracts.

I accept all contributions as ordered by importance and scientometric criteria. The candidate's contributions in the above directions demonstrate high professional competence in the field of medico-biological research, interdisciplinary knowledge with application in practice.

I have followed with great content the professional development of Assoc. Prof. Kiselova - from her entry as a PhD student in the Department of Biochemistry, Molecular Biology and Nutrigenomics to her transformation into a competent teacher, recognized scientist, institutionally committed and responsible person. On a personal level, Assoc. Prof. Kiselova is distinguished by modesty, correctness, delicacy, professional integrity and responsibility.

#### **6. Conclusion**

Assoc. Prof. Yoana Kiselova-Kaneva is an established scientist and teacher of biochemistry with extensive experience and practical skills. The analysis of her scientific output, as well as my personal impressions of her professional development, gives me reason to assume that she meets all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its application and the Rules for the Development of the Academic Staff of the Medical University – Varna for employment academic position "Professor". I strongly recommend



to the esteemed jury to award assoc. prof. Yoana Dimitrova Kiselova-Kaneva the academic title "Professor" in the field of higher *education 4. Natural sciences, mathematics and informatics*, professional direction *4.3. Biological sciences* and scientific specialty "*Biochemistry*", for the needs of the "Biochemistry, Molecular Biology and Nutrigenomics" Department, Faculty of "Pharmacy" at the Medical University, Varna.

November 30, 2022

Reviewer:



/Prof. Diana Ivanova, PhD, DSc/