R E V I E W

by Dr. Iliyan Ivanov Ivanov, Professor at the Department of Organic Chemistry, University of Plovdiv "Paisii Hilendarski "

of the materials submitted for participation in the competition

for the academic position of '***Associate Professor***'

at the Institute of Organic Chemistry with the Center for Phytochemistry (IOCCF), BAS

in the field of higher education	4. Natural sciences, mathematics and informatics,
professional field	4.2. Chemical sciences,
scientific specialty	"Bioorganic chemistry, chemistry of natural and physiologically active substances "

In the competition for an *Associate professor*, announced in the State Gazette, issue 53 from 25.06.2021 and on the website of IOCCF, BAS, as a candidate participates Chief Assistant Professor Dr. Manol Hristov Ognyanov from the Institute of Organic Chemistry with the Center for Phytochemistry at the Bulgarian Academy of Sciences, Laboratory "Biologically Active Substances – branch Plovdiv", Plovdiv.

1. General characteristics of the received materials.

Only a candidate has submitted documents for participation in the announced competition: Chief Assistant Professor Dr. Manol Hristov Ognyanov from the Institute of Organic Chemistry with the Center for Phytochemistry at the Bulgarian Academy of Sciences, Laboratory "Biologically Active Substances - Plovdiv", Plovdiv.

The set of materials presented by Dr. Manol Hristov Ognyanov is in full compliance with the Regulations for the development of the academic staff of IOCCF- BAS, and meets the criteria of IOCCF-BAS for the academic position of associate professor.

The candidate Chief Assistant Professor Dr. Manol Hristov Ognyanov has submitted a total of 20 scientific papers and a list of 27 research papers. 20 scientific papers that are outside the dissertation and are considered in the final evaluation and 25 research projects are accepted for review. Two of the presented projects are rather educational. The distribution of scientific papers on the respective Q factors is as follows Q1 - six, Q2 - four, Q3 - one and in editions with SJR without IF - nine. Five of the publications are presented as habilitation work (105 points, respectively from three publications in Q1 editions, one Q2 and one with SJR without IF) and fifteen in section G of the reference (230 points, respectively from three publications in Q1 editions, three in Q2, one in Q3 and eight with SJR without IF).

The observed citations of the scientific papers presented in the competition at the time of submission of the documents are 109 (Hirsch index 7). The total number of citations observed on all scientific papers is 202, respectively Hirsch index 10.

With the pointed indicators, the materials presented by Chief Assistant Professor Dr. Ognyanov fully meet and to some extent exceed the national minimum requirements (according to the LDASRB), and the requirements in the regulations for the conditions and the order for acquiring scientific degrees and for holding academic positions in the Institute of Organic Chemistry with Center for Phytochemistry, BAS.

2. Brief CV, education and professional experience.

Manol Ognyanov was born on November 20, 1985, in the city of Varna. In 2004 he graduated from the Vocational School of Tourism "Prof. Dr. As. Zlatarov" in the city, and in 2009 University of Food Technologies - Plovdiv, Bachelor's degree, specialty Biotechnology with professional qualification - engineer. In 2010 he obtained a master's degree at the same university, again with a degree in Biotechnology. After a successful full-time doctorate at the Institute of Organic Chemistry with the Center for Phytochemistry - BAS, Sofia, in 2016 he defended his dissertation in the doctoral program Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances on the topic: "Preparation and enzymatic modification of biologically active pectin polysaccharides", with supervisors: Assoc. Prof. Dr. MG Krachanova and Prof. Dr. St. D. Simova.

Dr. Ognyanov began his research activity in 2009 as a process engineer in the Laboratory of Biologically Active Substances - Plovdiv at IOCCF with research, development and implementation of innovative technologies and methodologies for functional foods and food additives. In the period 2010-2019 he held consecutive positions of engineer-technologist (researcher) and chemist-analyst in "ITC-Innovative-Technological Center" Ltd., Plovdiv. Since February 2014 he has been appointed to the academic position of assistant, and since June 2017 he has been elected to the academic position of Chief assistant at IOCCF at BAS, laboratory of "Biologically active substances - Plovdiv". In the academic year 2017/2018 he was a part-time lecturer at UFT-Plovdiv in the discipline of Biochemistry.

3. Evaluation of scientific activity.

Scientific publications, Habilitation work, Projects, Scientific forums.

The materials presented at the competition by Dr. Ognyanov demonstrate intensive and fruitful scientific and scientific-applied activity in current areas of bioorganic chemistry and chemistry of natural substances. The candidate's scientific research is in the field of studying the composition and properties of polysaccharides. One of the directions in the research is related to the study of the composition, structure and biological properties of polysaccharides isolated from medicinal plants, economically significant plant species and wastes from their processing. The second main direction in the scientific research of the candidate is the study of the chemical composition and antioxidant activity of medicinal plants, berries and vegetables, and the discovering of the possibilities for their application.

Indicative of the quality and timeliness of the research and the results achieved is the acceptance of the latter for publication in renowned journals - six of the scientific publications are

in scientific journals in Q1 and four in Q2. The intensity of the candidate's work is remarkable - the conducted research and their publication among the scientific community is within five years. The high citation rate of the reported results for the relatively short period of their announcement makes an excellent impression. The latter is another proof of the quality and the relevance of the research conducted by the candidate.

Given the nature of Dr. Ognyanov's research work, teamwork and the lack of independent scientific publications are completely understandable. Of the twenty publications presented, in five the candidate is the first author, in two issues in Q1, he is the first author.

The participation of Dr. Ognyanov in scientific and applied research projects directly related to his scientific interests is enviable. Twenty-seven projects, in two of which he is a head of the team. The projects are financed by various state and European institutions, and the share of projects of scientific and applied nature financed by business is significant. Two of the projects are related to the training of students funded by EU Operational Programs.

Recognition of the authority of Dr. Ognyanov among the scientific community is the intensive review activity. For a short period (2016-2021) he was invited more than 100 times as a reviewer by reputable publishers (Elsevier, MDPI, De Gruyter, Taylor & Francis, Wiley, OA Journals, RCS, etc.) for renowned journals - Carbohydrate Polymers, Biopolymers Research, Molecules, Food Chemistry, Biomacromole-cules, Polumers, Trends in Food Science and Technology, Industrial Crops and Products, Food & Function etc.

Educational and pedagogical activity of the candidate includes: tutoring of laboratory practicals in Biochemistry, part-time at UFT (2017-2018); tutoring of two diploma thesis graduates (students from UFT Plovdiv, Bachelor's and Master's degree); participation as a mentor in the project "Student Internships - Phase 1" and head of undergraduate internship of students from UFT-Plovdiv. The relatively modest educational and pedagogical activity is obviously a result of the opportunities provided by his place of work.

Contributions (scientific, scientific-applied, applied) and citations

The scientific research of Chief Assistant Professor Dr. M. Ognyanov is developing in two main areas closely related to the activities of LBAC - Plovdiv, in this regard the study of the composition, functional and biological properties of polysaccharides isolated from economically important plant species, medicinal plants and waste from their processing, and study of the chemical composition and antioxidant activity of medicinal plants, berries, and vegetables.

Dr. M. Ognyanov presents five publications as equivalent to a habilitation thesis - three in Q1, one in Q2 and one in an edition with SJR without IF. At first glance, the studied objects seem randomly selected - Mursal tea, leeks, callus cell cultures obtained from fumaria, locust bean flour and residues after freon extraction and hydrodistillation of calendula. All of them are united around the isolation and study of the composition, structure, functional and biological properties of the polysaccharides contained in them.

For the first time the quantitative content of polysaccharides in one of the most useful Bulgarian herbs - Mursal tea was evaluated. Cellulose and pectin have been found to be the main carbohydrates in the plant. An acid-soluble polysaccharide fraction representing a high molecular weight pectin polysaccharide was isolated. Through multi-enzyme fingerprinting and subsequent analysis of oligomers with modern analytical methods, information was obtained about its homolacturonan fragments. The obtained results are the basis for future evaluation of the

biological properties and its practical application as a stabilizer and emulsifier. (Carbohydrate Polymers 260 (2021) 117798)

A similar study was used in the study of acid-extracted leek polysaccharide. Galactose and galacturonic acid have been found to be the major monosaccharides in the polysaccharide fraction. By applying state-of-the-art analytical methods and approaches (LC_HILIC_MS and MALDI_TOF_MS) for the study of oligomers, it was found that rhamnogalacturonane segments are composed of regions not substituted by neutral sugar side chains and of highly cross-linked β–(1→4)–galactan chains with different degrees of polymerization. Accumulation of rhamnogalacturonane oligomers of the species $(Rha\text{-}GalA)_4$ was observed for the first time. Rheological studies of polysaccharide solutions were performed. It has been suggested that it should be used alone or in combination with other ingredients in emulsion-based foods - mayonnaise, dressings, etc. (Carbohydrate Polymers 2020)

In connection with the utilization of waste and useful components in them, as a potential source of polysaccharides, the potential of residues obtained after extraction with freon and hydrodistillation of calendula was evaluated for the first time. By extraction it was found that richer in polysaccharide is the raw material treated with freon - 9.8% and 3.1%, respectively. It has been shown that the type of processing of the raw material affects the properties and yield of the polysaccharide. It was found that in the raw material treated with freon, the isolated polysaccharide is highly esterified and contains a larger amount of uronic acids. Its ability to form healthy gels in the presence of sucrose and its viscoelastic properties are comparable to those of 0.5% citrus pectin. (Food Hydrocolloids, 2020)

A polysaccharide from an *in vitro* cultured cell culture of *Fumaria officinalis L.* has been successfully isolated and characterized. It was found that the isolated polysaccharide is composed mainly of homologacturonane segments with a mass of 6-600 kDa, weakly esterified (45%), with a degree of acetylation of 3.4%. Good foaming activity and emulsifying properties of a fraction with a concentration of up to 1% are shown, a prerequisite for its practical application as an emulsifier and stabilizer in food and medicinal products. (International Journal of Polymer Science, 2018)

In studies performed on organic locust bean flour (*Ceratonia siliqua L.*), a polysaccharide characterized as galatomanan (M / G = 3.5) was isolated. Two polysaccharide fractions with a molecular weight of 1724 kDa and 665 kDa, respectively, were isolated. Good water solubility (84%) and better oil retention than water retention capacity have been found. Locust bean flour is rated as rich in dietary fiber (galactomannan), low in fat and rich in polyphenols with well-defined antioxidant activity, which is the reason for its use as an ingredient in the production of functional foods. (J. Pharm. Sci. & Res. Vol., 2017)

A parallel study of three plant species widely used in ethnomedicine was carried out – above ground parts of purslane (*Portulaca oleracea L.*) and flowers of linden (*Tilia tomentosa Moench*) and lavender (*Lavandula angustifolia Mill.*). The chemical composition and immunomodulatory effects of three pectin-type polysaccharides were isolated and characterized. Linden-blossom pectin polysaccharides were studied for the first time and the chemical and biological activity of lavender polysaccharides was characterized. Like purslane, lavender and silver linden have been shown to contain immunomodulatory polysaccharides that may be useful in compromised immune systems. Studies on lavender polysaccharides have been extended to

study their chemical nature and immunomodulatory properties by isolating two highly esterified pectin polysaccharides composed predominantly of homolacturonan regions and a smaller proportion of rhamnogalacturonan-I fragments. Both fractions have been shown to have anti-inflammatory and intestinal immunomodulatory activity and may find real application in supporting the suppressed immune system or in controlling inflammation.

The second main direction in the research activity of Dr. Ognyanov is related to the study of the phytochemical composition, stability and antioxidant activity of economically important fruits, vegetables, medicinal and essential oil plants. Scientific research extends to a wide range of objects - rosehip, aronia, cornelian cherry, whitebeam fruits, pepper, medicinal plants (nettle, tansy, bladder campion, water pepper, common centaury, as well as waste from the essential oil and brewing industry – defatted milk thistle, rose, lavender, lemon balm, basil, yeasts).

Logically, the scientific research in the field of Chief Assistant Professor M. Ognyanov is related to the main object of research in LBAC-Plovdiv, the fruits of aronia. The research is led by Prof. Dr. Petko Denev and is related to the implementation of tasks related to a project funded by the NSF. The research has been developed in several directions. Study of the chemical composition of aronia, as a result of which it was found that aronia samples differ in both content and composition of organic acids, sugars and phenolic compounds. The differences in the chemical composition of the fruit allows to obtain functional foods that differ in chemical composition and antioxidant activity. It has been demonstrated that the temperature during juice pressing and nectar extraction has a significant effect on the content of polyphenolic components. The second direction is the search for approaches to their stabilization. Co-pigmentation has been used as one of the main mechanisms for stabilizing anthocyanins. Co-pigmentation is accompanied by an increase in color intensity and a decrease in color hue, associated with a more pleasing color. The best effect was observed with the use of rosemary acid, syringic acid and catechin. Eight herbal extracts were tested for their co-pigmentation ability with chokeberry anthocyanins. The use of herbal extracts leads to a significant hyperchromic effect at much lower co-pigment / pigment ratios compared to pure compounds. The application of herbal extracts as co-pigments makes it possible to develop functional chokeberry foods with improved organoleptic properties and biological effects, as a result of increased color stability and anthocyanins in them. The effect of γ-radiation on the chemical composition and antioxidant activity of dried chokeberry fruits was studied. The effect of radiation on dried chokeberry fruits was studied for the first time. As a result, it was found that irradiation increases the amount of total sugars and anthocyanins, reduces the content of organic acids and has no significant effect on polyphenolic content and antioxidant activity. By applying encapsulated in alginate gel juice of chokeberry and blackberry, a functional milk dessert enriched with polyphenolic compounds was created. The effect of alginic acid on polyphenols in a real food product was evaluated.

The fruits of rosehip are of scientific interest. For the first time a method and scheme for obtaining alcohol-insoluble part of cell walls was developed, and the monosaccharide composition was established. Tincture and aqueous extracts of rosehip were obtained by infusion, decoction and microwave treatment. The content of carotenoids, tocopherols, macro- and microelements in the starting plant material was determined, and the content of organic acids and carbohydrates was determined in the various extracts. It is stated that the best results in terms of soluble sugars and uronic acid content showed decoctions. In parallel with the study of rosehip, it

has been demonstrated that the medicinal plants nettle, tansy, bladder campion, water pepper and common centaury and extracts from them are a source of biologically active substances such as polysaccharides, macro- and microelements and vitamins. Studies have been conducted on "tea" from rosehip and rosehip containing herbal mixtures. As a result of the study, it was found that infusions show a higher content of decoctions, but the decoction allows better extraction of the contained polysaccharides. The study also has a clear practical focus on tea and soft drink producers.

The phytochemical and morphometric characteristics and the antioxidant potential of fresh, dried and processed cornelian cherry have been studied. It has been found that fresh and canned fruit has the best nutritional characteristics. The possibility of using their juice to enrich a dairy dessert with phenolic substances after its encapsulation in alginate pearls is shown.

The fruits of whitebeam fruits (Sorbus aria) as part of a study of forest fruit plants are another object of study by Dr. Ognyanov. The established low content of free sugars, the high content of dietary fibers and phenols are a prerequisite for their application as a healthy and dietary diet and the production of functional foods.

A study covering 63 genotypes, varieties, native forms and F1 pepper hybrids divided into five groups was performed, including characterization of chemical composition and antioxidant activity. The obtained results testify to the differences in the chemical composition both between the separate varietal groups and between the separate representatives in the groups.

Part of the research of Chief Assistant Professor M. Ognyanov are focused on the study of the possibilities for the use of "waste" from the essential oil and brewing industries as a raw material source of useful biologically active compounds. Residues after processing of milk thistle, oil rose, lavender, lemon balm, basil have been studied. It has been found that the partially defatted residue of milk thistle seeds is a rich source of silymarin, polyphenolic compounds, polysacchalides and protein, which is a prerequisite for its use as a food supplement or hepatoprotective functional food. It has been shown that from residues from the essential oil and aromatic industry (two from white oil-bearing rose and one each from lavender, lemon balm, common basil), polyphenols and aromatic components rich in water-alcohol extracts can be obtained, which can be used as biopreservatives or additives that increase the antioxidant capacity of food systems.

A different study is related to the successful application of an aqueous extract of waste obtained from the processing of oil-bearing rose and pasteurized / unpasteurized waste brewer's yeast to obtain silver nanoparticles.

Implementation activity

The materials presented by the candidate in the competition clearly show the practical orientation of the research conducted by him. In any case, the possibility for application of the achieved results in practice is clearly outlined - mainly in the field of food industry, production of functional foods, pharmacy.

4.Evaluation of the personal contribution of the candidate

From the submitted documents I cannot make a direct assessment of the personal contribution of the candidate in the respective publications and to what extent the formulated contributions and results obtained are his personal merit. I accept that the phytochemical studies

are the work of Dr. Ognyanov, while the biological ones are mostly with collaboration of his co-authors.

5. Critical remarks and recommendations

I have no critical remarks about the candidate. I wish Dr. Ognyanov to keep the intensity and quality of his scientific research.

6. Personal impressions

I do not know the candidate in the competition personally, but from the presented documents I have excellent impressions of the scientific and scientifically applied activity of Chief Assistant Professor Dr. Manol Ognyanov.

CONCLUSION

The documents and materials submitted by Chief Assistant Professor Dr. Manol Ognyanov **meet all the requirements** of the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for implementation of the Law on the Development of Academic Staff of the BAS and the Regulations for Development of the Academic Staff of IOCCF.

The candidate has submitted a sufficient number of scientific papers published after the materials used in the defense of the Educational and Scientific Degree Doctor. In the works of the candidate there are original scientific and applied contributions, which have received international recognition by being published in renowned journals referred to the Web of Knowledge and/or SCOPUS. The scientific qualification of Dr. Manol Ognyanov is unquestionable.

Achieved by Chief Assistant Professor Dr. Manol Ognyanov results in research activities are in full compliance with the specific requirements of the Regulations for the development of the academic staff of IOCCF.

After getting acquainted with the materials and scientific papers presented in the competition, analysis of their significance and contained in them scientific, scientific-applied and applied contributions, I find it reasonable to give my **positive assessment** and **recommend to the Scientific Jury** to prepare a report-proposal to the Scientific Council of IOCCF-BAS for election of Chief Assistant Professor Dr. Manol Ognyanov to the academic position of Associate Professor at IOCCF-BAS in the professional field 4.2. Chemical sciences, scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active substances".

October 28, 2021 Reviewer:

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Prof. Dr. Ilian Ivanov