

REVIEW

by Assoc. Prof. Dr. Zlatka Miltcheva Alexieva
Stefan Angelov Institute of Microbiology, BAS

Subject: The scientific production of Ch. Assistant Professor, Dr. Andrey Stoyanov Marchev for participation in a competition for the academic position "Associate Professor" for the needs of the Laboratory "Metabolomics", Department of Biotechnology, IMicB-BAS in professional field 5.11. Biotechnology. The competition was announced in State Gazette no. 12 of 12.02.2021

Dr. Andrey Stoyanov Marchev, Ch. Assistant Professor in the Laboratory "Metabolomics" at the Department of Biotechnology at the Department of Microbiology, BAS is the only candidate who submitted documents for the competition. The documents are presented in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules for its implementation in the Institute of Microbiology - BAS.

PROFESSIONAL BIOGRAPHY

Andrey Marchev has graduated at the University of Food Technology, Plovdiv, obtaining a Bachelor's degree in Biotechnology in 2005 and a Master's degree in Biotechnology in 2006. Proceed as an assistant at the Institute of Microbiology of BAS in 2012. Acquired the scientific and educational degree "Doctor" at the Institute of Microbiology "Stefan Angelov" - BAS in 2014, and in 2015 was elected and appointed as Chief Assistant at the Institute. Since 2017 he has held the position of Chief Assistant in the established Center for Plant System Biology and Biotechnology, Plovdiv.

Dr. Andrey Marchev works in the field of plant biotechnology and in particular conducts research on the biosynthetic capacity of plant in vitro systems for the purpose of biosynthesis of biologically active molecules. He has established himself as a specialist and applies metabolomics research based on nuclear-magnetic resonance (NMR) and high performance liquid chromatography (HPLC). The aim is to identification and quantification of the active molecules in complex plant extracts.

Dr. Andrey Marchev has conducted a number of specializations in prestigious research centers: Technical University of Dresden, Faculty of Mechanical Engineering, Institute of Food Processing, Dresden, Germany, 2010-2011; - Laboratory of Molecular and Cellular Biology of Cancer (LBMCC), Luxembourg, 2014; - Department of Cancer Research, Institute for Medical Research, Jacqui Tree Cancer Center, University of Dundee, Nine wells Hospital and Medical School, Dundee, Scotland, 2015; - Technical University of Dresden, Faculty of

Mathematics and Natural Sciences, Institute of Botany, Germany, 2015; - Technical University of Dresden, Faculty of Mathematics and Natural Sciences, Institute of Botany, Germany, 2017. The topics of these specializations have a significant impact on his development as a scientist with a wide range of views and approaches to research related to anti-inflammatory, antitumor and cytoprotective activity of secondary metabolites of plant origin, as well as the application of various approaches to metabolic engineering.

The participation of the candidate in research projects is also significant. The list contains 12 projects, of which 2 international and 10 national research projects. Andrey Marchev is the leader of three of these projects. The total amount of attracted funds for ImikB-BAS under the projects managed by Ch. Dr. Andrey S. Marchev, PhD, reaches to BGN 30,000. His scientific activity has been presented with 25 participations in international scientific forums (2 reports and 23 poster presentations at international scientific forums organized in Bulgaria, Greece, China, Croatia, Romania, and the Netherlands). He is a member of the organizing committee of three international scientific conferences. He received 4 awards for best poster.

Dr. Andrey Marchev has been a reviewer of a dissertation at the University of Alicante, Spain, of projects under the Program for Support of Young Scientists at the Bulgarian Academy of Sciences and has been an invited reviewer in over 10 peer-reviewed scientific journals. He is a member of the USB and of the Bulgarian Phytochemical Association, where he has been a Member of the Management Board since 2017.

MAIN SCIENTIFIC INDICATORS

The analysis of the data related to the indicators under the Minimal National Requirements of the Regulations for application of the Law on the Development of the Academic Staff in the Republic of Bulgaria shows the following:

50 points have been determined for the possession of Educational and Scientific Degree "Doctor". According to indicators from group B 10 publications are presented: 5 with Q1; 4 - with Q2; and 1 - with Q3, which gives an amount of 102.74 points. According to the indicators from group G, within indicator 7 are presented 17 publications, of which 8 - with Q1; 2 publication with Q2; 2 publication with Q3; 1 publication with Q4 and 4 in edition with SJR without IF. According to indicator 8, 11 publications are included, in not referred journals with scientific review or in edited collective volumes, and according to indicator 9 - 4 chapters of books. These data give a total of 202.70 points.

From the indicator for citations of publications in world-famous databases with scientific information (Web of Science and Scopus) or for indicators D in total, 218 citations without auto-citations are presented, which collect 2 190 points. Management and participation in research projects and the funds attracted from them, included in group E, gives the total number of 216 points.

The total number of points from the data filled in the Table for the minimum national requirements is 2 761.44 with a minimum required 400 t.

The conclusion is that Dr. Andrey Marchev overfulfills the requirements for each of the groups of indicators required to hold the scientific position of "Associate Professor" and generally scores many times (nearly 7X) more points than the mandatory minimum.

Regarding "Additional criteria for the development of the academic staff in IMicB" it can also be noted that the criteria are over fulfilled. For all his scientific activity, Andrey Marchev is an author in 49 scientific papers with a total IF 86.83, a total number of citations - 271 and H-index = 9.

For the competition, Dr. Andrey Marchev has presented 43 publications in journals with IF (excluding those included for "doctor"), in 17 of them the first and / or corresponding author. The required values for IF and H-index are many times exceeded. The additional requirement for participation and management of research projects has been exceeded.

SCIENTIFIC CONTRIBUTIONS

A significant place in the presented scientific production is occupied by research with medicinal plants. One of the main objects of research are representatives of the genus *Rhodiola* and mainly *Rhodiola rosea* (Golden Root, Golden Root). This species has highly recognized adaptogenic properties and is intensively used in traditional medicine as well as in clinical practice. In order to determine the phytochemical variations of the main secondary metabolites from different morphological parts of *R. rosea* Wild grown in Bulgaria 1H NMR-based metabolomics, combined with multivariate data analyzes, followed by the sensitive HPLC method. The aromatic compounds salidroside and rosavins are identified only in rhizomes and roots and are high enough to meet the pharmacopoeias criteria in countries such as Russia and the United States.

The use of a combined platform based on HPLC-UV and one-dimensional (1D) and two-dimensional (2D) NMR-based metabolomics has enabled integrated analysis of extracts of different types of *Rhodiola*, known and unique molecules, commercial products and the identification of impurities as well as the major metabolic differences, especially between *R.*

rosea and *R. crenulata*. The developed analytical approach can be applied at any stage of production of commercial products, starting with authentication and evaluation of raw materials to the finished product.

The potential of *R. rosea* extract and its main components salidroside, rosin, rosavin and rosin to alter cell growth of human Jurkat T cells, apoptosis of CD3 T cells in the spleen and expression of surface markers and phosphorylation of extracellular signal-regulated kinase (ERK) was also investigated. Data have been obtained showing that the different effects of rosin and rosavin on the expression of TRAIL (apoptosis-inducing ligand associated with tumor necrosis factor alpha TNF) may be due to effects on ERK. These results highlight their potential to manipulate TRAIL, which is important for the resistance to apoptosis in autoimmune diseases and cancer. The effect of pure salidroside, curcumin and their combination on the immunoreactivity of rats exposed to chronic mild stress (CMS) followed by lipopolysaccharide (LPS) -induced inflammation was studied. The synergistic effect of these compounds leads to a decrease in the levels of IL-6 and TNF- α , which shows their potential for the treatment of chronic stress and mild to moderate depression. NMR studies have shown that a standardized commercial rhodiola extract has a beneficial effect on learning and memory processes in naive rats and rats with memory impairment caused by scopolamine (9, 15, 19, 20, 21, 23).

Haberlea rhodopensis Friv. is an endemic for Bulgaria resurrected plant species, containing biologically active phenylethanoid glycosides, which have antioxidant activity. Metabolic profiling by magnetic resonance imaging based on in vitro cultured *H. rhodopensis* plants was performed, as well as the identification of molecules that increase the expression of a transcriptional antioxidant balance regulator (Nrf2) in bone marrow-isolated neutrophils. After fractionation, the fractions containing myconoside or myconoside and calceolarioside E and having the highest activity with respect to Nrf2 expression were determined. The therapeutic potential of both molecules for the regulation of pathological processes related to oxidative stress and the regulation of cellular homeostasis has been shown (8).

Phytochemical characterization of plant extracts of a poorly studied species of medicinal plant *Veronica austriaca* L., collected from two habitats in Bulgaria, was performed by NMR-based metabolomics and high performance liquid chromatography (HPLC). The glycoside arbutin was identified and quantified in both extracts as the major secondary metabolite. The effect of extracts and pure arbutin on the viability of neutrophils isolated from mouse bone marrow and cytokine production was studied by colorimetric analysis and flow cytometry (10).

In vitro pronounced anti-herpes activity of an aqueous extract of the plant *Nepetanudassp*, widely used in traditional medicine, has been proven. *nuda L.* on human herpes alphavirus (HHV). It is thought to affect both the early stages (absorption) and the late manifestations (replication) of HHV infection. The metabolic analysis of the extract shows as basic molecules rosemary, chlorogenic, gallic, vanilla, caffeic, protocatechuic, ferulic and cinnamic acids; while flavonoids are represented mainly by cirsimaritin, chryseoriol, vanillin, rutin and quercetin. (11).

The metabolic profile of *Clinopodium vulgare L.* (feline step) was analyzed. Caffeic and chlorogenic acids, catechin and other primary and secondary metabolites were identified by the application of 1D and 2D NMR profiling. The biological effect of *C. vulgare* extract (CVE) was studied in zymosan-induced COX-2 expression and apoptosis of murine neutrophils. The data obtained show that CVE has a pronounced potential to modulate neutrophil function and can serve as a source of new plant molecules with anti-inflammatory activity (14).

Much attention in research has been paid to transformed root crops (hairy roots, (HR)), obtained by infection with *Agrobacterium rhizogenes* and characterized by the multiplication of excessively branched roots. In-depth studies have been devoted to important components of secondary metabolism in members of the genus *Verbascum* (mulleins). NMR metabolomics has also been successfully used to determine the metabolic differences between the parent plant *Verbascum nigrum* and the transformed root crops obtained by genetic transformation with *Agrobacterium rhizogenes* (24).

A protocol was developed and for the first time a genetic transformation of *Verbascum eriophorum* (Lopen species) with *Agrobacterium rhizogenes* was performed. *Verbascum eriophorum* is a rare species with limited distribution and has been poorly studied in terms of phytochemical composition. Metabolic changes in the transformed root cultures relative to the mother plant were determined by 1D and 2D NMR-based metabolomics. As a result, the structure of phenylethanoid glycoside - verbascoside, which is the predominant metabolite in the transformed roots, was identified. These data indicate the possibility of using the obtained roots as a biotechnological platform for the sustainable production of valuable secondary metabolites of plant origin (22).

Interesting in this aspect is the elaboration for adapting the metabolism of hairy roots of tobacco for the production of stilbenes (t-resveratrol (t-R) in t-piceatanol (t-Pn) and t-pterostilbelene (t-Pt)). A metabolically engineered biotechnology system comprising a gene encoding stilbene synthase (STS) and / or transcription factor (TF) AtMYB12 was used to

generate a complete response in the phenylpropanoid synthesis pathway and coordinate the regulation of multiple metabolic steps. Artificial microRNA for chalcone synthase (amiRNA CHS) was used to stop normal fuchsia by the endogenous enzyme chalcone synthase (CHS). Transgenic HR is able to biosynthesize target stilbenes, but significant metabolic disturbances caused by TF AtMYB12 have been identified, confirming the complexity of biotechnological systems based on in vitro plant seed cultures for heterologous production of high value molecules (17).

As a summary, the scientific and scientific-applied contributions of Ch. Assistant Professor Dr. Andrey S. Marchev are related to several scientific fields, such as plant biotechnology, chemistry of natural molecules and pharmacology. The achievements are the result of the study of a fairly wide range of plant objects and methodological approaches of analysis, a significant part of which were described so far. On this basis, several conclusions might be formulated and highlighted:

1. In his research Dr. Andrey Marchev develops metabolic profiling for qualitative and quantitative analysis of secondary metabolites in medicinal plants, using the most modern methods such as nuclear magnetic resonance, high performance liquid chromatography, GC-MS, UV-VIS spectrometry, transmission electron microscopy, RT-PCR, etc.
2. The therapeutic potential of extracts of rare or protected plant species (*Haberlea rhodopensis* Friv., Representatives of the genus *Rhodiola*, *Veronica austriaca* L *Nepeta nuda* ssp. *Nuda* L. *Clinopodium vulgare* L., etc.) for the biosynthesis of valuable secondary metabolites (salidroside, curcumin, myconoside, calceolarioside E, etc.) has shown.
3. The cultivation of plant systems in vitro shows a real possibility for the production of valuable phytochemicals. By co-cultivation with *Agrobacterium rhizogenes*, the so-called hairy root crops were obtained, in which by transcriptional regulation of expression and inhibition of the synthesis of metabolic by-products, the metabolism of transgenic hairy roots of tobacco is adapted for the production of stilbenes (t-resveratrol and its derivatives).
4. A utility model has been developed and registered with a purpose to optimize the composition of the nutrient medium for in vitro propagation of the Orpheus flower (*Haberlea rhodopensis* Friv.).

5. An analytical approach has been developed for quality control of herbal preparations, through authentication and evaluation of raw materials, and monitoring of the stages of production of commercial preparations.

CONCLUSION

The works included for evaluation in this competition are distinguished by originality, topicality and public significance. Fully correspond to the scientific field of this competition. They are consolidated not only by the sought direct connection between basic and applied research but also by the skillful application and logical combination of a number of the most modern research methodological approaches. In addition, it should be emphasized that both a wide range of plant objects and changes in the function of genes associated with current medical problems have undergone metabolomic studies. The integration of plant in vitro systems and metabolomics for the study of cellular processes, gene regulation and biosynthesis of metabolites in controlled conditions leads to increased efficiency of biotechnological processes by cultivation of plant cultures, producers of biologically active substances of important pharmaceutical and medical importance. The obtained results reveal opportunities and perspectives for new research on current issues, focused on the needs of real biotechnological productions.

The scientometric data of Dr. Andrey Stoyanov Marchev significantly exceed the minimum national and additional requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, and the relevant Rules of IMiKB for holding the academic position "Associate Professor" in the Professional field 5.11. Biotechnology.

The achievements of Dr. Andrey Marchev have been published in authoritative scientific journals and have found a wide positive response in the scientific community in Bulgaria and around the world, have been reflected in a number of funded research projects, which undoubtedly confirms the importance of the tasks and applicability results.

All this gives me grounds to support the candidate and to convincingly recommend to the respected members of the Scientific Jury to evaluate positively and to propose to the Scientific council of IMiKB to award Ch. Assistant Professor Dr. Andrey Stoyanov Marchev, the academic position of "Associate Professor".

Sofia, June 7, 2021.

REVIEWER:

(Assoc. Prof. Dr. Zlatka Aleksieva)