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Report on the application for habilitation degree of Tomasz Tronina BEng. Ph.D.

Tomasz Tronina BEng. Ph.D. has been an assistant professor at the Department of Chemistry (currently Food Chemistry and Biocatalysis) at the University of Environmental and Life Sciences in Wrocław since 2013. Previously, he worked as a postdoctoral researcher at the Algatech Centre, Institute of Microbiology, Czech Academy of Sciences, Třeboň, Czech Republic, and as a research and teaching assistant at the Department of Chemistry at the University of Environmental and Life Sciences in Wrocław. Dr. Tronina has published about 30 scientific articles in the Journal Citation Reports database, of which 5 articles representing a thematic cycle in the field of biotechnology were selected for his scientific achievement. The cumulative impact factor of his publication is 99.071 and the total number of MEiN points is 5018. Based on Web of Science data, he has 605 citations and the Hirsch index is 16. With these qualifications, Dr. Tronina has submitted his application for habilitation degree in the field of natural sciences, in the discipline of biotechnology for his scientific achievement entitled “Biotransformations and biological activity of flavonoids from hops and Baikal Skullcap”.

All articles included in the scientific achievement were published as first author or corresponding author in renowned open access journals, 4 of them as corresponding author, two articles in the journal *Molecules* and the other three in the *International Journal of Molecular Sciences*. In these articles, Dr. Tronina's contribution includes the development of research hypotheses, the planning and execution of the experiments, the analysis of the data obtained, the writing of the scientific articles and the submission to the journals. In total, these articles account for a cumulative IF of 28.4 and 700 MEiN points.

The main objective of the research was the isolation and purification of flavonoids from hops (*Humulus lupulus* L) and Baikal skullcap (*Scutellaria baicalensis* Georgi), their biotransformation by filamentous fungi and the determination of the biological activity of the

resulting compounds. Publications 1, 2 and 4 of the scientific achievements concern the extraction and determination of the biological activity of hop flavonoids and their natural and chemical derivatives. Publication 3 reports on the purification and biotransformation of flavonoids from Baikal skullcap. Publication 5 presents research on biotechnological methods for the production of quercetin glycosides by cultures of entomopathogenic filamentous fungi. Overall, the scientific achievement focuses on the isolation, purification, biotransformation, chemical synthesis and bioactivity screening of flavonoids.

Flavonoids are an important group of compounds in plants. They are the products of phenylpropanoid pathway and play an important role in plant physiology and adaptation. Flavonoids are currently used in the functional food and cosmetic industries for their antioxidant and therapeutic properties. Xanthohumol is one of these flavonoids, which has anti-inflammatory, antioxidant, antibacterial, antifungal and antiviral properties and is effective against malaria, cancer, etc. One of the main sources of this compound is hops, which are used in the beer industry. Dr. Tronina has developed a method for extracting 98% pure xanthohumol from spent hop mash. Similarly, Dr. Tronina has developed a fast, inexpensive and efficient method to extract large amounts of the most active flavonoids, namely baicalein and wogonin, from Baikal skullcap, which is used in traditional Chinese medicine. Baikal skullcap is known for its antiviral effect against viruses that infect humans, including the SARS-CoV-2 coronavirus.

In addition, prenylated flavonoids such as α,β -dihydrochalcone and aurone have been produced from the pure xanthohumol to increase bioactivity. Although the presence of the prenyl group increases the biological activity of flavonoids, their non-polar nature reduces their bioavailability due to poor water solubility. Therefore, xanthohumol and its prenylated derivatives were used as substrates for biotransformation with filamentous fungal strains, which are known for their ability to catalyse a regioselective reaction to attach sugar residues to flavonoid molecules. Biotransformation of the three compounds led to the production of 6 new glycoside derivatives. Xanthohumol, α,β -dihydrochalcone and aurone and their glycosides were tested for various biological activities such as anti-inflammatory, serum albumin binding and cytotoxic activities. The results of these bioactivity studies are summarised in publications 1 and 4 of the scientific output. Similarly, the biotransformation of Baikal skullcap wogonin led to the production of the biologically active and rare flavonoid 4'-hydroxywogonin and two glucosylated wogonin derivatives, which are presented in publication 3. Besides the articles included in the scientific output, Dr. Tronina has published 25 articles in indexed journals as first author, corresponding author or co-author. Of the 30 articles listed, 25 articles were published after the PhD, including 21 articles as co-author, where he was involved in various aspects, such as participating in the experiments, analysing the data and contributing to the writing of the article.

Dr. Tronina has presented his findings at various national and international conferences. He has participated in 69 conferences, including 3 oral presentations. He has given 3 invited lectures and was a member and vice-chairman of the organising committee of two conferences. Of particular note is his participation as a member of the organising committee of the international conference organised by the FEBS (Federation of European Biochemical Societies) in Krakow in 2018.

He has completed three projects as Principal Investigator, including a major project funded by the National Centre for Research and Development and two other projects, including a SONATA project funded by the NCN. He has collaborated very well with industry. Two of his projects were carried out in collaboration with Healtcann S.A.. In one of these projects, the AdvanDropR technology was developed to dissolve insoluble lipophilic biologically active compounds from cannabis in water. The AdvanDropR technology was also the basis for the development of a formulation that allows lipophilic vitamins, in particular vitamins D3 and K2, to dissolve in water, significantly increasing their bioavailability. The AdvanDropR technology developed, for which a patent application has been filed, has been transferred to production and commercially available food supplements have been developed on its basis. Another project was carried out in collaboration with Greenvit Sp. zoo. to develop methods for the determination of prenylated hop flavonoids in raw materials and finished products based on conventional hops using UPLC. His scientific work has so far led to 61 approved national patents and 20 patent applications. One of these applications, "Composition containing cannabinoids", was subsequently filed for international protection (PCT/PL2021/050035).

He is a member of many scientific societies such as the Federation of European Biochemical Societies (FEBS), the Polish Chemical Society (PTChem), the Polish Society of Food Technologists (PTTŻ) and the Polish Biochemical Society (PTBioch). Dr Tronina was mobile and carried out his scientific activities at various foreign institutions. From 04.03.2013 - 30.06.2015 he worked as a postdoctoral researcher at the Institute of Microbiology, Algatech Centre, Czech Academy of Sciences. Under the scientific supervision of Professor Josef Komenda, he conducted research entitled "The Structure and Origin of Modified Products of the Tetrapyrrole Biosynthetic Pathway Excreted by Cyanobacteria". During this time he also completed a scientific internship at the Department of Biology, Washington University, (Saint Louis, USA), as part of the above-mentioned project under the supervision of Professor Robert Blankenship.

Dr. Tronina's scientific achievement entitled: "Biotransformations and biological activity of flavonoids from hops and Baikal Skullcap" is an important contribution to the development of the scientific discipline of biotechnology. Dr. Tronina's scientific achievements, other scientific achievements, as well as his activities in the field of scientific cooperation, which he has carried out in various institutions, teaching and popularisation of science, indicate his significant scientific and research independence. Having examined the documents, I confirm

that Dr. Tronina is a mature and experienced scientist and meets the requirements for candidates applying for the doctor habilitated degree in accordance with Article 219 section 1 point 2 and 3 of the Act of July 20, 2018, Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended). I give my positive opinion on the application of Dr. Tronina for the award of the academic degree of habilitated doctor in the field of natural sciences with specialisation in biotechnology.