



THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

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Characterization of the selected honeybee products based on omics techniques

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ABSTRACT

The research project "Characterization of the selected honeybee products based on omics techniques" is to comprehensively characterize honeybee venom, royal jelly, propolis, and pollen, by applying advanced analytical and bioinformatics methodologies. Honeybee products (HBP) contain many bioactive components with both beneficial and harmful effects on the human organism. Nevertheless, the overall composition of the HBP remains not fully investigated. Thus, this research is focused on complementary proteomic and metabolomic characterization of biologically active compounds derived from HBP, regarding their toxicological and pharmacological properties. The objectives of the study will be achieved by the application of up to date mass spectrometry techniques. Due to increasing interest in using of HBP in medicine, this project will contribute to improving the safety of HBP-derived dietary supplements and drugs.

Keywords: honeybee products, proteomics, metabolomics, mass spectrometry.

General Information

The research project entitled „Characterization of the selected honeybee products based on omics techniques" was awarded by the Polish National Science Center (NCN) in the SONATA12 competition under grant number 2016/23/D/NZ7/03949. The project is run by Department of Inorganic and Analytical Chemistry at Poznan University of Medical Sciences, Poland. The principal investigator is Jan Matysiak (Ph.D.). The duration of the grant is 36 months, from 29th August 2017 to 28th August 2020.

This proposed study will contribute to broadening the knowledge of the honeybee products (HBP), such as honeybee venom, royal jelly, propolis, and pollen. It is known, that HBP contain many bioactive components with potential biological, toxic or allergic properties [1–3]. Because of their beneficial effects on the human organism, HBP have been used in medicine since ancient times. The present-day medical field utilizing HBP in the treatment of various diseases is termed apitherapy and is gaining popularity [4]. Nevertheless, despite increasing interest in natural honeybee-derived products, the composition and

the molecular basis of HBP activities remain not fully characterized. This project, using innovative approaches with the most modern LC-MS (mass spectrometry-liquid chromatography) techniques, will result in the discovery of new biologically and pharmacologically active compounds. Moreover, it will contribute to the evaluation of the biological activity of HBP. This is of great significance for assessing the quality and safety of HBP-based products (dietary supplements and drugs). Thus, this research will positively affect healthcare, improving human wellness.

Finance

This project is mainly financed by a grant from the NCN Life Science Panel in the SONATA12 competition. The total grant value is 1,199,810 Polish Zloty. Grant funds were earmarked to purchase new laboratory instrumentation, disposable laboratory equipment and reagents for the conducting of proposed research. In particular, MALDI Imaging Equipment is planned to be purchased for MALDI imaging studies. Also, grant funds were designed to cover personnel costs of project participants and costs associated with the promotion of research results and publications in reviewed journals.

Research project objectives

This research is focused on complementary proteomic and metabolomic characterization of biologically active compounds derived from HBP (honeybee venom, royal jelly, propolis, and pollen). There are three main hypotheses in this project:

- › Proteomic and metabolomic study of HBP will lead to the discovery of new compounds with biological activity.
- › HBP contain compounds with toxic properties (their presence and concentration are related to environmental contamination in HBP sampling locations) that impact on the specific biological activity of HBP.
- › The applied bioanalytical methods will allow gaining detailed knowledge of the biological activity of the studied compounds. That will

contribute to the explanation of the influence of HBP on the human organism.

The objectives of the study will be achieved by the application of up to date mass spectrometry techniques supported by advanced bioinformatics tools. Mass spectrometry is a modern technique that deals with the identification and studying of the structure of the chemical compound. In this proposed research, novel methodologies based both on nanoLC-MALDI-TOF-MS (nano-liquid chromatography-matrix assisted laser desorption/ionization-time of flight mass spectrometry) and LC-ESI-QTOF-MS (liquid chromatography-electrospray ionization-hybrid quadrupole-time of flight mass spectrometry) will be developed. Mass spectrometry coupled with liquid chromatography allows for detection and identification of thousands of low-abundance molecules in biological matrices. Thus, applying complex targeted and untargeted proteomic and metabolomic approach will lead to the discovery of new biologically active HBP components. Moreover, the proposed strategy will enable to assess the variability of the composition of HBP. Factors responsible for that variability will be pointed out, as a biological activity of HBP depends on their constituents.

Quality of HBP is not only associated with their composition. The contaminants (i.e., pesticides, organic pollutants, pharmaceutical residues, and heavy metals) may significantly weaken the properties of bee-derived products. In particular, accumulation of pesticides in HBP should be examined in terms of their toxic effect both on humans and on bees [5]. Therefore, this study aims to evaluate the impact of the contaminants on HBP and to assess exposure of honeybees to various chemicals. The multi-compound screening methods enabling simultaneous analysis of miscellaneous groups of pollutants will be developed.

As mentioned before, the constituents of HBP, due to different biological properties, may affect the functioning of the human organism. To investigate the mechanism of HBP activity and effects on live organisms, the bioassay analysis will be performed. According to the literature, the insect model was successfully applied in the characterization of the mechanism underlying human diseases [6–8]. Therefore, in this study insect tissues will be used instead of those

derived from humans, as the insect tissues' physiology is considered to be similar to the human organism. Therefore, the aim of this project is to explore potential pharmacological and toxic properties of HBP and their constituents by complementary use of bioassay and mass spectrometry analysis. The alternative to the classical MS techniques is mass spectrometry imaging (MSI). MSI coupled with matrix assisted laser desorption/ionization (MSI-MALDI) has been previously reported in honeybee venom compounds monitoring in envenomed tissues [9]. Thus, in this project, novel MSI-MALDI procedures will be implemented.

Research plan and basic concept

The main goal of this project is to comprehensively characterize natural HBP by applying advanced analytical and bioinformatics methodologies. The HBP may be a valuable source of components with potential biological activity. Proteomic and metabolomic techniques will allow for detection of novel low molecular compounds (including amino acids), peptides and proteins which can be used in medicine. To date, most of the reported studies were focused on honeybee venom investigations [1, 2, 10]. Therefore, in order to extend knowledge of HBP, within this project, large-scale omics research will be conducted. In particular, the study will focus on:

- › Collecting of HBP and bee samples and creation of biorepository in which the samples and data will be stored. Materials and data retained in biorepository could be used for future investigations.
- › Development of innovative methods based on modern analytical techniques. These methodologies may be implemented to the characterization of HBP as well as other natural products.
- › Compilation of data obtained from proteomic, metabolomic, toxicological and bioassay analyses.
- › Creation of a database of HBP and their constituents. This database will consider biological, toxicological and pharmacological properties of the HBP. It will collect the results of this research and could be extended by results obtained from other projects.

Research methodology

The research will be conducted using HBP samples collected from 4 apiaries located in Poland. During three years, from each apiary, three samples of each HBP type will be collected within four months of each year. Samples will be stored at -80°C in a newly created biorepository. In order to perform comprehensive analyses, two mass spectrometers coupled with liquid chromatography systems will be used: nanoLC-MALDI-TOF-MS and LC-ESI-QTOF-MS. The combination of multiple strategies will ensure the complete insight into HBP. Thus, in this study, the application of both targeted and untargeted mass spectrometry-based methodologies is planned. The project is planned for 36 months, and the main assumptions include the following tasks:

- › Development of toxicological methods and study of toxicology. To isolate the HBP compounds, different sample preparation strategies will be tested, such a lyophilization, solid phase extraction (SPE), homogenization, liquid-liquid extraction (LLC) and QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe). The targeted analysis will be performed using LC-ESI-QqQ-MS (hybrid triple quadrupole-linear ion trap mass spectrometer) system. For untargeted analysis, LC-ESI-QTOF-MS will be used.
- › Development of bioassay methods and study of bioassays. Different isolation methods (isoelectric focusing, liquid chromatography, gel electrophoresis, capillary electrophoresis) for HBP components will be tested. Studied substances derived from HBP will be injected into insect samples. The mass spectrometry analyses will be performed using MSI-MALDI system.
- › Development of proteomic methods and proteomic studies. To achieve proteomics purposes of this project, for the protein-peptide profiling of HBP compounds, MALDI-TOF-MS and ESI-QTOF-MS systems will be used. New protocols for HBP analysis will be developed. Moreover, because of the sensitivity of mass spectrometry analyses, various sample pretreatment strategies will be tested, like SPE, isoelectric focusing, two-dimensional gel electrophoresis, and combinatorial ligand library. Additionally, isobaric labeling for semi-quantitative analysis will be implemented.

- › Development of metabolomic methods and metabolomic studies. Untargeted and targeted metabolomic studies will be performed by using the hyphenated LC-MS techniques. LC-ESI-QTOF-MS and nanoLC-MALDI-TOF-MS platforms will be used for investigation of HBP components.
- › Data analysis and omics data compilation. The results obtained from HBP investigations will be statistically analyzed by univariate and multivariate approaches. Correlation analyses between the obtained results and pathway analyses will be performed. The data will be interpreted using various databases. Statistical analysis will enable to assess the variability in HBP composition and the responsible factors.
- › HBP database constitution. Creation of a new open access database of HBP and their constituents, regarding their biological, toxicological and pharmacological properties is planned. This database could be used in future HBP studies.

Measurable effects and expected results

This project will result in the deep characterization of HBP. We expect, that applying combined proteomic and metabolomic strategies, including mass spectrometry techniques, allows to significantly extend the knowledge of HBP composition and their biological properties. Due to increasing interest in using of HBP in medicine, this project will contribute to improving the safety of HBP-derived dietary supplements and drugs. Moreover, a compilation of the obtained data will result in a better understanding of the molecular mechanism of HBP activities. The impact of the contaminants (in particular pesticides) on the HBP properties will also be assessed. In addition, the development of novel advanced omics strategies and creation of unique HBP database will allow to comprehensively investigate the HBP and other natural products in the future.

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Conflict of interest statement

The authors declare no conflict of interest.

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References

1. Matysiak J, Hajduk J, Mayer F, Hebel R, Kokot ZJ. Hyphenated LC-MALDI-ToF/ToF and LC-ESI-QToF approach in proteomic characterization of honeybee venom. *J Pharm Biomed Anal.* 2016;121:69–76.
2. Matysiak J, Hajduk J, Pietrzak Ł, Schmelzer CEH, Kokot ZJ. Shotgun proteome analysis of honeybee venom using targeted enrichment strategies. *Toxicol.* 2014;90:255–64.
3. Matysiak J, Schmelzer CEH, Neubert RHH, Kokot ZJ. Characterization of honeybee venom by MALDI-TOF and nanoESI-QqTOF mass spectrometry. *J Pharm Biomed Anal.* 2011;54(2):273–8.
4. Kim CMH. Apitherapy – Bee Venom Therapy. In: Grassberger M, Sherman RA, Gileva OS, Kim CMH, Mumcuoglu KY, editors. *Biotherapy – History, Principles and Practice.* Dordrecht: Springer Netherlands; 2013. p. 77–112.
5. Sánchez-Bayo F, Goulson D, Pennacchio F, Nazzi F, Goka K, Desneux N. Are bee diseases linked to pesticides? – A brief review. *Environ Int.* 2016;89–90:7–11.
6. Ugur B, Chen K, Bellen HJ. *Drosophila* tools and assays for the study of human diseases. *Dis Model Mech.* 2016;9(3):235–44.
7. Alfa RW, Kim SK. Using *Drosophila* to discover mechanisms underlying type 2 diabetes. *Dis Model Mech.* 2016;9(4):365–76.
8. Tabunoki H, Bono H, Ito K, Yokoyama T. Can the silkworm (*Bombyx mori*) be used as a human disease model? *Drug Discov Ther.* 2016;10(1):3–8.
9. Francese S, Lambardi D, Mastrobuoni G, la Marca G, Moneti G, Turillazzi S. Detection of Honeybee Venom in Envenomed Tissues by Direct MALDI MSI. *J Am Soc Mass Spectrom.* 2009;20(1):112–23.
10. Kokot ZJ, Matysiak J, Urbaniak B, Dereziński P. New CZE-DAD method for honeybee venom analysis and standardization of the product. *Anal Bioanal Chem.* 2011;399(7):2487–94.

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