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Evaluation Report on PhD Thesis by Maciej Korczak

Strategies of utilizing bioactive potential of natural products-derived postbiotic metabolites

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Legal basis for assessment

The assessment was prepared on the basis of the invitation letter of a chairman of the Pharmaceutical Sciences Discipline Council, prof. Grzegorz Nałęcz-Jawecki; RND/RDNF/D-760/2/24; Medical University of Warsaw, Poland.

Formal aspect

MPharm Maciej Korczak submitted the doctoral thesis dealing with the chemical and pharmacological studies of postbiotic products of plant origin. The structure of the PhD thesis is well and correctly presented, consists of 11 chapters. At the beginning of this dissertation list of publications, table of contents, list of figures, list of abbreviations, abstracts, scientific profile of the doctoral candidate, introduction, aim of the thesis are described. In the next part the thesis summary and conclusions, co-authors' statement, references and reprint of publications constituting the doctoral dissertation are presented. The thesis is written on 244 pages altogether, including 82 pages as a general description and enriched by figures, schemes, and tables. The theoretical principles as well as the research part were validated with 113 valuable references, 43% were published over 2020, what means that Maciej Korczak over the last few years was regularly updating his knowledge in this field.

Overall merit of the PhD thesis and scientific goals

Plant-based metabolites play an important role in health care systems, however, the state of research on the activity of natural compounds is still incomplete. From the therapeutic viewpoint plant based-drugs are of great importance and have multiple-applications. One of the most intensely studied areas of research has been the investigation of plants with well-recognized use in traditional medicine. The beneficial effects of plants secondary metabolites on human health and well-being are widely recognized. The nowadays medicine is to a large part developed from traditional knowledge, so time to look again with the new tools to our ancestors' knowledge. Evaluation of plant metabolites requires application of variety of highly selective analytical techniques able to deal with complex mixtures of compounds with quite different physical characteristics. Nowadays a shift to an investigation of single compounds is being extensively observed, including *in vitro* and *in vivo* tests. A new strategy is to research an activity of natural products-derived postbiotic metabolites, which allows for understanding their mechanisms of action, establishing pharmacokinetic parameters and predicting their applicable potential.

MPharm Maciej Korczak in his research was mainly focused on plants compounds being exposed to an action of human microbiota and consequently on researching new metabolites. Therefore, as the main objective of doctoral dissertation he chose a study of so-called postbiotic metabolites and their potential application to the skin as an anti-inflammatory agent. He focused on urolithin A and its derivatives, next on the stability and safety tests, and finally the tests in an animal model, choosing rats. The last set of investigations constitutes a part of the LIDER project and connects science with market demand.

The goals include three strategies that represent a new approach to research postbiotic metabolites derived from plant products. From these strategies it is seen that the Author has a defined view of the entire project and how to successfully complete all tasks. In my opinion the aims of the work were formulated in a clear and logical manner. The topic is interesting for all scientists working on plant-based drugs and contributes to the general knowledge about natural products.



Methodology

The research shows a multidisciplinary character and includes a wide array of methodologies (i.e. development of chemical synthesis of metabolites, identification of metabolites, anti-inflammatory activity study). The methodology used is described in detail and is relevant to the research questions as well as is adequate and justified. The articles of the thesis, especially a review's one, reflect sufficient competence in the survey of literature and documentation of statements. The Author demonstrates good erudition and vast knowledge in the field of interest.

In an experimental part MPharm Maciej Korczak did apply modern analytical approaches, used high quality tools in order to do all research. Such techniques as NMR spectroscopy, UHPLC-DAD-MS, Western blot, or permeability assays in Caco-2 cells are worth of a special attention and their apply demonstrates a high level of research advancement. These tools meet all criteria in current pre-clinical pharmaceutical sciences. In the pharmacological part of investigations negative or positive controls were used, which gives reliable comparisons.

Scientific merit of the PhD thesis

The dissertation insists of 3 publications, one review and two original ones.

In the review the Author introduced a detailed state-of-art in the subject, this manuscript reviews the history of natural drugs, plant-derived drugs, new perspectives within phytochemical studies, and finally describes postbiotic metabolites and their bioactivity. It is evidence that the Author deeply understood the subject, theoretical knowledge and problem.

In the next two original articles the Author described the methods and techniques used in the experimental section. The Author presents the results that confirm hypothesis and then discuss them with those available in literature. From these two publications it clearly results that in a scientific activity of the Author, two research trends can be distinguished. The first one includes the chemical analysis with synthesis and the latter includes the pharmacological tests.

The Author for the first time has synthesized 4 compounds, derivatives of urolithin A with 4 nonsteroidal anti-inflammatory drugs (ibuprofen, mefenamic acid, diclofenac, acetylsalicylic acid). Additionally, the Author confirmed their anti-inflammatory activity in *in vitro* models, proving that mefenamic acid derivative had the strongest impact on a reduction of inflammation. Simultaneously, this derivative exhibited high stability in human plasma and for this reason was chosen for further studies. These results mean that mefenamic acid-urolithin A derivative has a significant anti-inflammatory activity and potential to be, in the future, used in reducing of inflammation as a single drug or in synergistic drug combinations. These findings contribute to a general knowledge about plant-based postbiotic compounds and, may be a reason for consideration to use the results in commercialization by plant-based drugs manufacturers.

This part is well written and documented, there are not typing errors, the text is written in clear manner. All results are presented in a logical manner, easy to read and are included in tables. The conclusions confirm that the formed hypotheses of the work were successfully proven.



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Originality and innovation

The candidate presents a contribution to the knowledge of the bacterial metabolites and their potential use in the prevention or treatment of skin diseases. The great value is combining the chemical experiments with pharmacological examination of postbiotic metabolites in order to reveal their anti-inflammatory activity. The thesis as a whole is original as a concept, describes comprehensive approach to the research problem and provides new insights into mechanism of action of postbiotic metabolites and their synthetic derivatives.

The great value is a practical application of the obtained results. Such an approach has resulted in a patent application to the Patent Office of the Republic of Poland, which reflects a high market demand on these groups of postbiotic metabolites. In reviewer's opinion, it also means that the results are outstanding and have a world-class character. Because science should serve the entire society, the results obtained here are intended to increase the quality of life, especially amongst people affected by chronic inflammation. The project results, in a form of new urolithin A derivatives can be applied for other industries like cosmetics, what makes the innovation may have a wider array of application.

However, there is one remark, which has been raised during reading the dissertation:

* in a botanical name of the plants, the name of the author of the genus in some places is missing, e.g. Linnaeus; L.; it should appear at least by the first mention the plant. If it is stated, the lowercase is used (*Hypericum perforatum* L., *Valeriana officinalis* L., etc.).

Final conclusion and evaluation statement

The suggestions mentioned above do not change my positive opinion. The doctoral dissertation meets all required criteria i.e.:

- a) has an original character and provides an original solution to a research problem, all research is of an international standard,
- b) has a practical aspect, what meets criteria of Industry 5.0 (Gospodarka 5.0) of the Polish Government,
- c) confirms that the Author is the independent researcher as well as proved by Author's publications in which he is the first Author.

In conclusion, I can state that the PhD thesis meets all requirements for obtaining PhD according to the Act of July 20, 2018, Higher Education Law and Science (with later amendments). The thesis presents original research of great importance and is ready to be defended orally, in front of respective committee.

On the basis of the above statements, I would like to propose to reward the doctoral dissertation due to the following aspects: novelty, a high quality and extensive range of research, a large contribution to the plant-based postbiotic compounds and a scientific confirmation of anti-inflammatory activity of urolithin A.

Respectfully,

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