OPINION

by **Assoc. Prof. Dimitar Georgiev Bozhilov, PhD – Associate Professor at Plovdiv University "Paisii Hilendarski", Faculty of Chemistry, Department of Organic Chemistry**

on the dissertation thesis for awarding the educational and scientific degree **"Doctor"**

in: Field of Higher Education: **4. Natural Sciences, Mathematics and Informatics**

Professional field: **4.3. Biological Sciences**

Doctoral program: **Biochemistry**

Author: Angel Iliev Peshkov

Topic: "Study of the Properties of Oxidoreductases Immobilized in Biocompatible Matrices"

Supervisors:

1. Prof. Ilia Nikolov Iliev, PhD – Plovdiv University "Paisii Hilendarski", Faculty of Biology
2. Assoc. Prof. Nina Dimitrova Dimcheva, PhD – Plovdiv University "Paisii Hilendarski", Faculty of Chemistry

1. General presentation of the procedure and the doctoral candidate

By Order № RD 22-1277 dated 27.05.2025 of the Rector of Plovdiv University "Paisii Hilendarski" (PU), I was appointed as a member of the scientific jury for the defense procedure of Mr. Angel Iliev Peshkov's dissertation thesis on the topic "Study of the Properties of Oxidoreductases Immobilized in Biocompatible Matrices" for obtaining the educational and scientific degree "Doctor." The set of materials submitted by the doctoral candidate on electronic media complies with Art. 36 (1) of the Regulations for the Development of the Academic Staff of PU.

2. Relevance of the topic

The topic of the dissertation is relevant and up to date, as it addresses the problem of environmental pollution and the need to develop "greener" and more environmentally friendly technologies. The research focuses on a large class of enzymes – oxidoreductases – which are of great importance in bioremediation, industry, and medicine. The dissertation includes important directions:

- Environmental pollution: The dissertation points out that increasing environmental pollution with various toxic compounds (e.g., phenolic compounds, which are harmful to health) requires the search for effective and economically viable removal methods.
- Innovative and eco-friendly technologies: The use of enzymes such as laccase and catalase in biosensors provides an alternative to traditional physico-chemical methods, which are often more expensive and generate secondary pollutants.
- Development of biosensors: The study of immobilized enzymes for biosensor development in pollutant analysis is a modern and rapidly developing field. In this case, biosensors for the analysis of

di- and trihydroxyphenolic compounds were developed.

- Application in phytochemistry: The work presents original results, such as the development of an electrochemical method for monitoring the catalytic activity of immobilized catalase and the application of a biosensor method for determining the total phenolic content in herbal extracts.

3. Familiarity with the problem

The doctoral candidate has gained deep insight into the problem, as confirmed by the literature review, which is rich in information, well systematized, and stylistically sound. The biochemical and electrochemical methods used are adequate for achieving the stated goals. The approach is comprehensive – from enzyme extraction and purification to their immobilization, electrochemical characterization, and evaluation of their activity under different conditions.

4. Research methodology

The analytical methods are described in detail, which ensures reproducibility of the experiments. The research includes both biochemical and electrochemical methods, demonstrating its multidisciplinary character.

5. Characteristics and evaluation of the dissertation and contributions

The dissertation consists of 137 pages and contains 1 scheme, 28 figures, 14 tables, and 246 cited literature sources. A classical structure is used, including: literature review, formulated goals and tasks, methodology, results and discussion, conclusions and contributions, as well as a reference list. A significant number of experiments were conducted, which guarantees the reliability of the obtained data. The results are presented clearly and visibly. The contributions are mainly scientific but also possess application potential, especially in the characterization of herbs and products derived from them.

6. Evaluation of publications and the doctoral candidate's personal contribution

On the topic of the dissertation, 3 articles have been published in refereed journals – Ecologia Balkanica (Q4), Applied Food Biotechnology (Q3), and Open Chemistry (Q3). According to Bulgarian legislation (ZRASRB), the national minimum requirements for obtaining the scientific degree "Doctor" amount to 30 points. The candidate's scientometric indicators exceed this minimum, totaling 42 points. The results of the dissertation research have been presented at 5 international and 4 national conferences. The doctoral candidate is the first author in two of the articles, which gives me grounds to conclude that he has made a substantial contribution not only to the research itself but also to its presentation.

7. Abstract

The abstract is 36 pages long and contains 12 tables and 16 figures, which accurately reflect the main results and contributions of the dissertation and comply with the specific requirements of Plovdiv University "Paisii Hilendarski."

8. Recommendations for future use of the dissertation contributions and results

I have the following comments and recommendations for the doctoral candidate:

1. On page 15, there is an incomplete sentence, which hinders the understanding of the context.
2. In Figure 4, the structural formulas of naproxen and diclofenac are missing, which limits the completeness of the presented information.
3. A description of the herbs from which the extracts were prepared is missing, including information on the method of analysis of these extracts.
4. The text states that the extracts were analyzed by HPLC, but the detector used, the chromatographic conditions, and the method for determining total phenolic content are not specified.

The above notes and recommendations do not diminish the scientific and applied value of the dissertation and do not affect its essence. They are formulated with the aim of improving the candidate's work and supporting his future scientific research.

Conclusion

The dissertation of Angel Iliev Peshkov contains scientific and applied results with original contributions, which meet the requirements of the Bulgarian legislation (ZRASRB), its application regulations, and the regulations of Plovdiv University "Paisii Hilendarski." The doctoral candidate possesses profound theoretical knowledge and skills for independent scientific research in the field of electrochemistry and biochemistry.

I give a positive evaluation of the dissertation thesis and propose to the esteemed scientific jury to award the educational and scientific degree "Doctor" to Angel Iliev Peshkov in the field of Natural Sciences, professional field 4.3. Biological Sciences, Doctoral Program "Biochemistry."

08.08. 2025 г. **Prepared by**: .....

signature

/ Assoc. Prof Dimitar Bozhilov, PhD /