STATEMENT OF OPINION

by Detelina Stoyanova Belkinova, PhD,

Associate Professor at the Department of Botany and Biological Education,

Faculty of Biology, Paisii Hilendarski University of Plovdiv

of a Dissertation for Obtaining the Educational and Scientific Degree "PhD"

Field of higher education: 4. Natural Sciences, Mathematics and Informatics

Professional direction: 4.3. Biological Sciences

PhD Program Botany

Author of the PhD Thesis: Tsvetelina Georgieva Andonova

Topic: Phytochemical and Biological Studies on the Invasive for the Bulgarian Flora Plant Species

Ailanthus altissima (MILL) SWINGLE and Koelreuteria paniculata LAXM.

Scientific supervisors:

Prof. Dr. Ivanka Zhecheva Dimitrova-Dyulgerova - Paisii Hilendarski University of Plovdiv

Assoc. Prof. Dr. Iliya Zhelev Slavov - Medical University, Varna

1. General Presentation of the Procedure and the PhD Candidate

With Order No. PД-21-1788/08.11.2022 of the Rector of *Paisii Hilendarski* University of Plovdiv, I was assigned as a Member of the Scientific Jury for provision of procedures of dissertation defence on the following topic: Phytochemical and Biological Studies on the Invasive for the Bulgarian Flora Plant Species *Ailanthus altissima* (MILL) SWINGLE and *Koelreuteria paniculata* LAXM., for obtaining the educational and scientific degree 'PhD'. The author of the dissertation is Tsvetelina Georgieva Andonova - PhD candidate, full-time mode of education at the Department of Botany and Biological Education.

The set of materials on the procedure, provided by Tsvetelina Andonova, is under Art. 36 (1) of the Regulations for the Development of the Academic Staff of the University of Plovdiv and includes all the necessary documents.

Biographic and Professional Reference for the PhD candidate

Tsvetelina Andonova completed her higher education at the University of Plovdiv (1995-2000), where she acquired the Educational and Qualification Degree 'Master', with major in Microbiology and Genetics. In 2002, she was hired as a laboratory specialist at Vinprom Peshtera AD, the town of Plovdiv. In 2015, she was hired at the position of a biologist, at the Departments of Biochemistry and Microbiology, of Zoology, and of Physiology of Plants and Molecular Biology, consequentially, and, since 2021 to this moment, she has been working as a biologist at the Department of Botany and Biological Education of the Faculty of Biology of the University of Plovdiv. On 1 March 2019, Tsvetelina Andonova was enrolled as a regular PhD candidate and after successful performance of the activities and tasks from her individual plan of education, in 2022, she was discharged with the right of defence.

2. Actuality of the Topic

In the past few decades, the interest of the scientific community to invasive species has been increasing, as they have become a major problem of the terrestrial and aquatic habitats. It is known that when invasive species invade new territories, they are able to cause irreversible changes in the environment by suppressing the local species, changing the structure of the food networks, or reducing the diversity. Both tree species, *Ailanthus altissima* and *Koelreuteria paniculata* are foreign for the Bulgarian flora and have a significant invasive potential. However, like most plants, they contain natural compounds with biological activities and potential positive effects on human health. Their phytochemical characteristics have not been completely studied yet, as for Bulgaria, by 2019, developments on their chemical composition and biological activities were almost missing. This specifies the actuality of this dissertation.

3. Awareness of the Problem

The literature review is thorough and provides detailed information on the level of phytochemical study of both species – globally and in Bulgaria. The available information is very well structured, in three subsections: 1.1 Brief botanical characteristics of *A. altissima* and *K paniculata*; 1.2 Literature data on the phytochemical composition (primary, secondary metabolites) and biological activities of *A. altissima*; 1.3 Literature data on the phytochemical composition (primary, secondary metabolites) and biological activities of *K. paniculata*. The literature reference skilfully leads to the purpose and objectives of the dissertation.

4. Methods of Study

In accordance with one of the major objectives of the dissertation (study of seasonal dynamics of accumulation of the main classes of phenolic compounds), rich plant material is used, collected during the vegetation season of three consecutive years. The selected methods of study are on a contemporary scientific level, with high quality of preciseness: quantitative spectrophotometric methods, performed under proven international standards; methods of phytochemical screening (high-performance liquid chromatography, gas chromatographic/mass spectrometric analysis); methods of specifying biological activities (agar-diffusion method, antioxidant DPPH, ABTS, CUPRAC and FRAP analyses, DNA protection potential - supercoiled plasmid DNA test, etc.) and statistical methods.

5. Characteristics and Assessment of the Dissertation and the Contributions

The thesis presented by Tsvetelina Andonova consists of 148 pages, 21 tables, and 25 figures and it follows the standard structure in the following sections: Introduction (2 pages), Literature Review (25 pages), Purposes and Objectives (1 page), Materials and Methods (15 pages), Results and Discussion (68 pages), Conclusions (3 pages), and Literature Cited (147 sources: 1 in Cyrillic and 146 in Latin).

The dissertation has a clearly specified purpose - to do a phytochemical screening and study the biological activity of plant substances of *A. altissima* and *K. paniculata*. The obtained results are illustrated in an excellent way and are with the necessary level of reliability. The conclusions synthesize the obtained results on all the specified objectives. They are similarly formulated for both species, which makes it easier to compare. The contributions are divided into original scientific contributions and applied scientific contributions. It is worth paying special attention to the study of the seasonal dynamics of the accumulation of the secondary metabolites in the plant substances of *K. paniculata* and *A. altissima* and to the study of the phenolic profile of ethanolic extracts of aerial plant substances of *K. paniculata*, through HPLC analysis. What

is an innovation for the science is also the identification of main diagnostic microscopic features of powdered plant substances from vegetative and generative organs of *K. paniculata*, and from flowers of *A. altissima*. The specified chemical composition of essential oils of *K. paniculata* and of fatty seed oils of both species have applied scientific significance, as future possibilities are studied for their application in foods, in cosmetic, and medicinal products.

6. Publications Connected to the Dissertation

In connection to the dissertation, two scientific publications, which **leading author** is the PhD candidate, have been attached to the materials on the procedure. The articles have been published in the renowned journals *Plants* (IF_{Q1}: 4.658) and *Journal of Essential Oil-Bearing Plants* (IF_{Q3}: 1.541), which proves the high level of the studies. Some of the results of the dissertation have been presented in two international conferences. This fact exceeds the minimal national criteria and the specific criteria of the Faculty of Biology for obtaining the Educational and Scientific Degree 'PhD'.

7. Abstract

The abstract has the correct structure and presents a true description of the content and the achieved results of dissertation.

8. Personal Impressions

I know Tsvetelina Andonova from our collaboration at the Department of Botany and Biological Education. Her working style excels with responsibility, propriety, and professionalism. In my opinion, the she did the experimental research on the dissertation herself, as I had the opportunity to watch her work in the laboratory, which she performed with great devotion, diligence, and determination. Now, when her dissertation is ready, I think that, under the competent supervision of her scientific supervisors, she has also managed to learn the skill of developing and forming a qualitative dissertation, with a number of original scientific and applied scientific contributions.

CONCLUSION

Tsvetelina Andonova's PhD thesis contains enough scientific and applied scientific results, which represent an original contribution in the science and meet all the requirements of according to the Act on the Development of the Academic Staff in the Republic of Bulgaria, ZRASRB, the Regulations for the Implementation of ZRASRB and the relevant Regulations of *Paisii Hilendarski* University of Plovdiv.

The presented data and fact give me a reason to be sure about the **POSITIVE** assessment of the developed dissertation and to **offer the honorable scientific jury to award Tsvetelina Georgieva Andonova the educational and scientific degree 'PhD'** in the following field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional direction 4.3. Biological Sciences, PhD program **Botany**.