

R E V I E W

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for awarding the higher education and scientific degree “Doctor” (Doctor of Philosophy, PhD) in the area of higher education **4. Natural Sciences, Mathematics and Computing**, professional field **4.3. Biological Sciences**, Doctoral programme in **Botany**

Candidate: Tsvetelina Georgieva Andonova

Thesis title: Phytochemical and Biological Studies on the Invasive for the Bulgarian Flora Plant Species *Ailanthus altissima* (Mill.) Swingle and *Koelreuteria paniculata* Laxm.

Thesis Supervisors: prof. Ivanka Zhecheva Dimitrova-Dyulgerova, PhD – Paisii Hilendarski University of Plovdiv and assoc. prof. Iliya Zhelev Slavov, PhD – Medical University -Varna, Prof. Dr. Paraskev Stoyanov

1. General description of the submitted materials

By order № RD-21-1788 from 08.11.2022 of the Rector of the University of Plovdiv Paisii Hilendarski (PU), I was appointed as a member of the of the academic board of examiners to provide a procedure for the defense of a dissertation entitled " 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 “Doctor” in the area of higher education 4. Natural Sciences, Mathematics and Computing, professional field 4.3. Biological Sciences, Doctoral programme in Botany. The author of the dissertation is Tsvetelina Georgieva Andonova – PhD student in full-time education at the Department of Botany and Biological Education, PU with supervisors prof. Ivanka Zhecheva Dimitrova-Dyulgerova, PhD from Paisii Hilendarski University of Plovdiv and assoc. prof. Iliya Zhelev Slavov, PhD from Medical University -Varna, Prof. Dr. Paraskev Stoyanov.

The set of paper materials presented by Tsvetelina Georgieva Andonova is in accordance with Art.36 (1) of the Regulations for development of the academic staff of the University of Plovdiv and includes the following documents:

- letter to request to the Rector of the University of Plovdiv for disclosure of the procedure for defense of the dissertation;

- curriculum vitae – EU template;
- protocol of the Department Council at the Department of Botany and Biological education, related to reporting on the readiness to open the procedure and preliminary discussion of the dissertation;
- PhD Thesis;
- PhD abstract (in Bulgarian);
- PhD abstract (in English);
- list of scientific publications on the topic of the dissertation;
- declaration of originality and authenticity of the attached documents;
- reference for compliance with the minimum national requirements;

All presented documents are prepared correctly and precisely and correspond to the requirements of PU.

2. Brief Biographical Data for the PhD-student

Tsvetelina Georgieva Andonova obtained the professional qualification "Biologist" and the educational-qualification degree "Master", professional qualification "biologist" with specialization in microbiology and genetics at the Faculty of Biology of the University of Plovdiv (PU) (1991 – 2000). She has been a member of the academic society of the Faculty of Biology - PU since 2008, holding the position of "biologist" successively in the departments of "Biochemistry and Microbiology", "Zoology", "Plant Physiology and Molecular Biology" and "Botany and Biological Education". The professional experience of Tsvetelina Andonova also includes her work as a laboratory assistant in the physicochemical and microbiological laboratories of "Vinprom Peshtera" JSC, Plovdiv (2002-2007). She was trained as a full-time doctoral student in the Botany doctoral programme at the Department of Botany and Biological Education.

3. Relevance of the Topic and Appropriateness of the Set Goals and Tasks

The study of plant species regarding their phytochemical composition and biologically active action, as a potential for the design of new medicinal products, is a direction in world scientific research with unceasing relevance, responding to the concept of health as a basic human right and a basic value of society. Invasive species are the focus of a number of modern studies in relation to their negative impact on local ecosystems and in a number of cases as a potential danger to human health. At the same time, modern science is turning its attention to

these species as alternative plant raw materials, which makes it possible to preserve the populations of valuable species of the native flora and supports a more ecological way of managing the populations of invasive species. The choice of objects and research directions in the presented dissertation completely fall into these current problems and are in accordance with modern approaches to their solution. For the two species *Ailanthus altissima* and *Koelreuteria paniculata* there are no comprehensive studies on the territory of Bulgaria, only fragmentary data are available. The set goal of studying the phytochemical composition and some biological activities of herbal substances of these species, as well as the set tasks of tracking the seasonal dynamics in the accumulation of secondary metabolites (phenolic compounds), chromatographic study of aerial plant parts, respectively ethanol extracts, essential oils and fatty oils (HPLC, GC/MS), *in vitro* study of biological activity (antimicrobial, antioxidant and antitumor) and DNA protection potential of extracts and microscopic characterization of powdered herbal substances. The set goals and tasks are precisely formulated and reflect the overall idea of the scientific research and, last but not least, allow their implementation within the period of the regular doctoral studies.

The stated facts outline the topic of the proposed research as current, timely and with the possibility of obtaining results both of a scientifically fundamental nature and of practical applied significance.

4. Understanding the Problem

The presented literature review covers a brief botanical description of the studied species (1.1), followed by literature data on the phytochemical composition and biological activities of *Koelreuteria paniculata* and *Ailanthus altissima* structured in two independent parts 1.2 and 1.3, respectively, which include: primary metabolites, secondary metabolites (phenolic compounds, terpenes and terpenoids, alkaloids, etc.), biological activities (antibacterial, antiviral, antifungal, antimalarial, antioxidant, antitumor, DNA protective activity and other activities) and other aspects of application of the species. The same approach was used in the presentation of both types, which allows the doctoral student to determine the goals and tasks of the research in a motivated manner. The overview information is built on literary sources, covering classic scientific publications and state-of-the-art scientific studies. The known data are presented correctly, in a logical sequence, including an analytical element.

It follows from the above that the doctoral student knows well the objects of research, known in the scientific literature on the problem, skillfully and analytically handles the scientific literature.

5. Research methodology

Classical methods of collection and chamber processing of the plant material were used. Voucher herbarium specimens were prepared and deposited, accompanied by the necessary data. Extracts were obtained from the collected plant material, with selected solvents corresponding to the tasks set in the study. The methods are described in detail, including sufficient information. The instrumental methods for the analysis of the extracts (SFM, HPLC, GC/MS, as well as the in vitro studies of biological activity are described in separate subchapters, indicating the used apparatus, reagents and methods for analyzing the results. Mass spectral data libraries (GMD, NIST'08) were used. When determining the quantitative composition and physicochemical characteristics, classical and pharmacopoeial methods of analysis (Ph Eur 10, GFR XI), approved methods of analysis (AOAS), as well as ISO standard procedures for glyceride oils were applied. Validated and modern methods for in vitro determination of biological activities of extracts such as agar-diffusion method for determination of antimicrobial activity, MTT test for antiproliferative activity, four tests for antioxidant activity (DPPH, ABTS, CUPRAC and FRAP), supercoil test are applied. plasmid DNA (pUC10) to determine protective activity of extracts against oxidative DNA damage, light microscopic analysis of herbal substances and statistical analyzes (ANOVA).

It is worth emphasizing the large number and variety of applied analysis methods. The selection of methods corresponds to the determined goal and the set tasks and is a prerequisite for an objective assessment of the species as a potential for pharmaceutical application.

6. Characteristics and Evaluation of the Dissertation

The presented dissertation consists of 148 pages of text, 21 tables, 25 figures (graphs, diagrams, photographic material, chromatograms, photomicrographs) and 8 appendices (tables and chromatograms – HPLC, GC/MS). The structure follows the requirements for the presentation of scientific research and includes the following main parts: Introduction (2 pages), Literature Review (25 pages), Aim and Tasks of the Dissertation (1 page), Material and Methods (15 pages), Results and Discussion (68 pages), Conclusions (3 pages), References (22 pages). A

declaration of originality is also included with the dissertation. The individual parts are correctly presented and balanced in terms of volume and proportion. The doctoral student motivates the choice of topic and object of the dissertation work, as an actual and unstudied problem for the territory of the country. It correctly defines the objective "to study the phytochemical composition and some biological activities of the herbal substances of *Koelreuteria paniculata* and *Ailanthus altissima*, foreign to the Bulgarian flora" and formulates the resulting tasks. The presented data on what has been published so far in the scientific literature corresponds in structure and way of presentation with the described results and discussion. Literary sources are correctly cited and analyzed. The dissertation is written in good scientific style, adhering to scientific terminology. The References list is precisely formed. The figures, tables and appendices accurately and clearly illustrate the presented results and are included in the main text, selected appropriately and structured according to the requirements of a scientific text. The list of references consists of 247 titles, of which 1 in Cyrillic and 246 in Latin, covering the period from 1957 to 2022 and is a comprehensive literature reference.

In the PhD thesis, sufficient volume and structure of material for the scientific research was used. Doctoral student Tsvetelina Andonova demonstrates skills in presenting experimental research in a scientific work, evidence of which is also the presented scientific publications.

7. Contributions and significance of development for science and practice

As a result of the research conducted, the summaries and conclusions drawn, the doctoral student formulated two groups of contributions: original scientific contributions and scientific contributions of an applied nature. For the first time, basic diagnostic microscopic features of powdered herbal substances from flowers, leaves and stem barks of *K. paniculata*, and flowers of *A. altissima* are indicated. For the first time, the seasonal dynamics in the accumulation of total water-soluble polyphenols, tannins, flavonoids and phenolic acids in herbal substances from *K. paniculata* and *A. altissima* (monitored over a 3-year period) were tracked. For the first time, volatile components from aerial parts of *K. paniculata* were isolated by water distillation and identified by GC/MS analysis. For the first time, the phenolic profile (flavonoids and phenolic acids) of ethanolic extracts of dry substances (flower buds, flowers, leaves and stem barks) of *K. paniculata* was studied by HPLC analysis. A phospholipid profile of fatty oils from *A. altissima* and *K. paniculata* seeds is reported for the first time. DNA-protective potential of ethanolic extracts of flowers, leaves and stem barks of *K. paniculata* as well as flowers and leaves of *A.*

altissima has been demonstrated for the first time. The conducted study shows the potential of the studied plants and herbal substances as a natural source for: (i) essential oils from *K. paniculata* and use in food, cosmetic and medicinal products; (ii) for *A. altissima* and *K. paniculata* seed fatty oils with a valuable composition of beneficial omega fatty acids, phospholipids, sterols and vitamin E and possible future applications; (iii) biologically active compounds in extracts of aerial parts of *K. paniculata* and *A. altissima* (especially flowers and leaves) as a good source of natural antioxidants.

The established chemical composition of the isolated essential oils from *K. paniculata* shows their potential as a natural source for ingredients in food, cosmetic and medicinal products. The studied composition of the fatty seed oils of both species showed that they are rich in beneficial omega fatty acids, phospholipids, sterols and vitamin E, making them a valuable source of these components with possible future applications. Due to the presence of valuable BAS in extracts of aerial herbal substances of *K. paniculata* and *A. altissima* (especially flowers and leaves), they would be a good source of natural antioxidants.

I accept the original scientific contributions and scientific contributions of an applied nature presented by the doctoral student.

8. Evaluation of the dissertation publications

In the materials for the defense of a dissertation, the doctoral student presents two (2) scientific articles with research results published in 2020 and 2021. The two articles were published in English and co-authored with a scientific team, with PhD student Tsvetelina Andonova as the first author. The publications reflect specific results achieved in the dissertation, which is proof of the contribution of the doctoral student. The two publications are in authoritative international scientific journals, referenced and indexed in the world scientific databases as follows: *Journal of Essential Oil-Bearing Plants* (Q3, SJR₂₀₂₀ 0.36, IF₂₀₂₀ 1.541) and *Plants* (Q1, SJR₂₀₂₁ 0.77, IF₂₀₂₁ 4.658).

4 positive citations were noted in the world scientific databases (Scopus) reflecting published results of the dissertation. Three of the citations are from foreign authors in authoritative scientific publications such as: *Microbial Pathogenesis* (IF₂₀₂₁ 3.843), *International Journal of Environmental Research and Public Health* (IF₂₀₂₁ 4.614) and *Journal of Environmental Management* (IF₂₀₂₁ 8.91). The citations are in publications in the fields of microbiology, environmental engineering, heavy metal accumulation and stress in plants, biological activities of extracts, etc., which

presents the research as an object of interest to a wide range of specialists in various scientific fields. The response time ≤ 1 year is proof of the relevance of the conducted research and high evaluation by the scientific community.

The research data are presented at the International Conference on Technics, Technologies and Education ICTTE, in 2020 and 2021, respectively.

9. Personal participation of the doctoral student

The submitted materials for the procedure, as well as the declaration submitted by the doctoral student for the originality of the presented data, show that the collection, camera processing of the plant material and laboratory research in the dissertation work and its writing were carried out with the personal participation of the doctoral student Tsvetelina Andonova. The presented dissertation, formulation of conclusions and contributions are personal merit.

10. Abstract

The abstract is structured according to the requirements and correctly reflects the main results achieved in the dissertation.

11. Critical remarks and recommendations

Single inaccuracies were noted, which essentially did not change the overall positive impression of the work and the results achieved.

12. Personal impressions

I know Tsvetelina Andonova as a doctoral student in the Department of Botany and Biological Education - PU. My personal impressions are from her publication activity, which presents her as a precise author and scientist.

It is worth noting that part of the period of full-time doctoral studies takes place during the imposed pandemic restrictions, although the work program is completed and the dissertation is submitted on time for defense, accompanied by high-quality publications. This is a fact that shows purposefulness, consistency and very good organization in the work of the doctoral student. Undoubtedly, credit for this goes to the scientific supervisors and the doctoral student's ability to work in a team.

13. Recommendations for future use of dissertation contributions and results

The present dissertation undoubtedly shows the potential of the species *Ailanthus altissima* and *Koelreuteria paniculata* and their populations in Bulgaria as a valuable natural source of biologically active compounds with the possibility of use in the prevention and treatment of a number of socially significant diseases. The presented study is a contribution to the metabolic profile of the two plants and a multi-target study on their biological activity. The acquired knowledge and skills of the doctoral student in this field are a prerequisite for deepening the studies on these species or the selection of other objects and natural products. It is of interest to expand the range of biological activities studied in the future, as well as to study them with different methods. The development of new and optimized methods for obtaining extracts is another opportunity to increase the potential of using the herbal substances of the species as an active ingredient in foods, nutritional supplements, cosmetic products and herbal medicinal products.

CONCLUSION

The dissertation contains scientific and scientific-applied results, which represent an original contribution to science and meet all the requirements of the Development of the Academic Staff in the Republic of Bulgaria Act (DASRBA), the Regulations for the Implementation of DASRBA and the relevant Regulations of University of Plovdiv Paisii Hilendarski (PU). The presented materials and dissertation results fully correspond to the specific requirements of the Faculty of Biology, adopted in connection with the Regulations of the PU for the application of the DASRBA.

The dissertation shows that the doctoral student Tsvetelina Georgieva Andonova possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Botany" by demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results and contributions, and **I propose to the esteemed scientific jury to award the educational and scientific degree "Doctor" to Tsvetelina Georgieva Andonova** in in the area of higher education 4. Natural Sciences, Mathematics and Computing, professional field 4.3. Biological Sciences, Doctoral programme in Botany.

29.11. 2022.

Sofia

Reviewer:

/Prof. Anely Metodieva Nedelcheva, PhD/