

REVIEW

by **Dimcho Zahariev Ivanov, PhD** – Professor in Bishop Konstantin Preslavsky
University of Shumen

of a Dissertation for awarding the educational and scientific degree "Doctor"

in the field of higher education: 4. Natural Sciences, Mathematics and Informatics

Professional field: 4.3. Biological Sciences

Doctoral program: *Botany*

Author: *Tsvetelina Georgieva Andonova*

Subject: *Phytochemical and biological studies on the invasive for the Bulgarian flora *Ailanthus altissima* (Mill.) Swingle and *Koelreuteria paniculata* Laxm.*

Scientific supervisors: *Prof. Ivanka Zhecheva Dimitrova-Dyulgerova, PhD - Paisiy Hilendarski University of Plovdiv, Assoc. Prof. Ilia Zhelev Slavov, PhD - Medical University "Prof. Dr. Paraskev Stoyanov" - Varna*

1. General description of the presented materials

By Order No.P21-1788/08.11.2022 of the Rector of Paisiy Hilendarski University of Plovdiv, I have been appointed as a member of the scientific jury to ensure a procedure for the defense of a dissertation on the subject: „**Phytochemical and biological studies on the invasive for the Bulgarian flora *Ailanthus altissima* (Mill.) Swingle and *Koelreuteria paniculata* Laxm.**“ for the acquisition of the educational and scientific degree "Doctor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, Doctoral program "Botany". The author of the dissertation is Tsvetelina Georgieva Andonova - full-time doctoral student at the Department of Botany and Biological Education with scientific supervisors: Prof. Ivanka Zhecheva Dimitrova-Dyulgerova, PhD - Paisiy Hilendarski University of Plovdiv, Assoc. Prof. Ilia Zhelev Slavov, PhD - Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

The set of paper materials presented by Tsvetelina Georgieva Andonova is in accordance with Art. 36 (1) of the Regulations for the Development of the Academic Staff of the Paisiy Hilendarski University of Plovdiv and includes the following documents:

- request to the Rector of Paisiy Hilendarski University of Plovdiv to disclose the procedure for the defense of a dissertation work;
- CV in European format;

- protocol from the departmental council related to reporting the readiness to open the procedure and preliminary discussion of the dissertation work;
- dissertation work;
- abstract - in Bulgarian and English;
- list of scientific publications on the subject of the dissertation;
- copies of scientific publications;
- list of noticed citations;
- declaration of originality and authenticity of the attached documents;
- certificate of compliance with the minimum national requirements for obtaining the educational and scientific degree "Doctor".

The doctoral student has attached 2 publications on the subject of the dissertation work.

2. Brief biographical data of the PhD student

Tsvetelina Georgieva Andonova received a master's degree in Microbiology and Genetics at the Faculty of Biology of the Paisiy Hilendarski University of Plovdiv in 2000. In the period April 2002 - May 2007, she worked as a laboratory technician in the "Physicochemical and Microbiological Laboratories" of "Vinprom Peshtera" AD - Plovdiv. From August 2008 to July 2014, she worked as a biologist at the Department of Biochemistry and Microbiology of Paisii Hilendarski University of Plovdiv. Since February 2015, she has been working as a biologist in the Department of Botany and Biological Education, Department of Zoology and Department of Plant Physiology and Molecular Biology of the Paisiy Hilendarski University of Plovdiv. Tsvetelina Andonova's work experience is long enough to acquire the necessary knowledge, skills and competencies for conducting biological experiments and working with scientific information.

3. Relevance of the topic and appropriateness of the set goals and tasks

In Bulgaria, invasive alien plant species are the subject of ecological studies, but they are underestimated as objects suitable for medicinal purposes. They are not used in official medicine, and their use in folk medicine is very limited. In our country, no research was conducted on the chemical composition and biological activities of species from this group of plants at the time of choosing the topic for the dissertation work. Exceptions are two studies, the first of which is a review of secondary metabolite content and biological activities of *A. altissima* based on data from studies conducted abroad (Kožuharova, E.; Lebanova, H.; Getov, I.; Benbassat, N.; Kochmarov, V. (2014). *Ailanthus altissima* (Mill.) Swingle - A terrible invasive pest in Bulgaria or potential useful medicinal plant? Rev. Pap. Bothalia, 44(3): 213–230) and study of the carotenoid fraction isolated from *K. paniculata* flowers (Zhelev, I., Georgiev, K., & Dimitrova-Dyulgerova, I. (2016). *In-vitro* antioxidant and

antineoplastic activities of carotenoids from flowers of *Koeleruteria paniculata*. World Journal of Pharmaceutical Research, 5(5): 53-60). The isolation and study of biologically active substances and the establishment of their biological activities may reveal new possibilities for the practical application of invasive alien plant species and help control their spread. That is why I believe that the purpose of the dissertation work is appropriately selected, relevant and meets the qualifications of Tsvetelina Andonova. The planned tasks enable the achievement of the goal, which is proven by the results obtained and the conclusions formulated.

4. Knowing the problem

The doctoral student demonstrates in-depth knowledge of the state of the problem set for research. This is evident both from the information presented in the literature review and from the discussion of the results obtained during the research. With the presented dissertation, she proves her ability for independent and in-depth scientific analysis. The work covers a significant number of literary sources on the subject, which are analyzed and skillfully used in the review of available information, in the selection of appropriate objects and methods for conducting research, and also in the interpretation of the obtained results.

5. Research methodology

The chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks solved in the dissertation work. The most modern methods for investigating the chemical composition were used: quantitative spectrophotometric methods for the determination of total water-soluble polyphenols, tannins, total phenolic acids and flavonoids (according to European Pharmacopoeia 10.0); HPLC analysis to determine the content of flavonoids and phenolic acids; gas chromatographic/mass spectrometric analysis of ethanolic extracts and essential oils from aerial plant parts; chemical, physicochemical and chromatographic methods for the analysis of the chemical composition of glyceride oils (according to the ISO 659:2014; ISO 12228-1:2014; ISO 9936:2016; ISO 10540-1:2014 and others).

The methods for *in vitro* study of biological activities of extracts are also appropriately selected: for antimicrobial activity – agar-diffusion method; for antitumor activity – MTT test against PC3 and HT-29 cell lines; for antioxidant activity – DPPH, ABTS, CUPRAC and FRAP analyses; for DNA protective potential – test with supercoiled plasmid DNA (pUC 19).

The light-microscopic analysis of powdered plant substances was carried out according to the European Pharmacopoeia 10.0.

It is impressive that the experimental part was carried out in a large number of laboratories: the scientific laboratories of 3 departments of the Faculty of the Paisiy Hilendarski University of Plovdiv; Applied Biotechnology Laboratory of the Stefan Angelov Institute of Microbiology at the BAS; the Institute of Experimental Morphology, Pathology and Anthropology with a museum at the BAS; Department "Technology of fats, essential oils, perfumery and cosmetics" at University of Food Technology - Plovdiv; Agrobio-Institute - Sofia at the Agricultural Academy.

6. Characterization and evaluation of the dissertation work

The presented dissertation has a volume of 148 pages. The structure includes the following sections: introduction, literature review, aim and objectives, material and methods, results and discussion, conclusions, statement of originality, literature, and appendix. The volume of each of the sections is appropriately selected and proportional to the overall volume of the dissertation.

A list of abbreviations used is included at the beginning of the dissertation, which is extremely useful. The introduction skillfully points to the subject of the dissertation work. The literature review presents a brief botanical description of the two species under study, as well as an overview of the available information on their phytochemical composition and biological activities. To achieve the set goal, seven specific tasks have been formulated. The materials and methods included in the research are appropriately selected and allow the implementation of the planned tasks. The results are presented systematically, they are well illustrated with tables (21 pcs.) and figures (25 pcs.) and are followed by a well-balanced discussion. As a result of the conducted research, 10 conclusions were formulated for each species, object of the study. They show that the goal and set tasks have been successfully completed. A declaration of originality of the results and contributions of the dissertation work is attached. The list of used literature includes 247 titles, of which 1 in Cyrillic and 246 in Latin. At the end of the thesis, the main results of the phytochemical analysis are systematically presented in 8 appendices in the form of 4 tables and 4 chromatograms.

7. Contributions and significance of the development for science and practice

Seven contributions of original scientific character can be defined. They are related to the establishment of: basic diagnostic microscopic features of powdered plant substances from different parts of plants of both species; the seasonal dynamics in the accumulation of a number of biologically active substances, followed for 3 years; isolation by water distillation and identification of volatile components from aerial parts of *K. paniculata*; the phenolic profile (flavonoids and phenolic acids) of ethanolic extracts of dry substances (flower buds, flowers, leaves and stem barks) of *K. paniculata* by HPLC analysis; the phytochemical composition and different biological activities (antimicrobial, antioxidant, antiproliferative) of ethanolic extracts of fresh plant substances of *A. altissima* and *K.*

paniculata; the phospholipid profile of fatty oils from *A. altissima* and *K. paniculata* seeds; The DNA-protective potential of ethanolic extracts of flowers, leaves and stem barks of *K. paniculata* and of flowers and leaves of *A. altissima*.

The scientific and applied contributions are three in number. The established composition of the essential oil of *K. paniculata*, of the fatty oils of *A. altissima* and *K. paniculata* and of the aerial parts of both species (especially flowers and leaves) makes them suitable for use in the food, cosmetic and pharmaceutical industries.

8. Assessment of dissertation publications

The presented materials include 2 articles published in full text in English in refereed international scientific journals, indexed in primary databases (Scopus and Web of Science). The first article is in a journal with quartile 3 (Q3) and IF=1.541, and the second article is in a journal with quartile 1 (Q1) and IF=4.658. In both articles, Tsvetelina Andonova is the first author. By number of authors, the first article has 5 authors, and the second has 6 authors. Since no separation protocols have been presented, I assume that the contribution of each of the authors is equal.

Both participations in international scientific conferences during the period of development of the dissertation make a good impression.

The interest generated by the published results can be judged by the four positive citations (according to data from Scopus). Both the two articles and one of the two reports presented at international scientific conferences are cited. This is impressive, especially considering the short period of time from the publication of the articles to the defense of the dissertation work for the award of the educational and scientific degree "Doctor".

9. Personal participation of the doctoral student

The personal participation of the doctoral student in the conducted dissertation research is indisputable. Suitable objects and methods for their research have been selected, which is proven by the successfully achieved goals and tasks. The doctoral student skillfully analyzes the obtained results and formulates accurate conclusions. The results of the good theoretical and practical preparation of the doctoral student are the contributions received from the dissertation work.

10. Abstract

The abstract is prepared according to the requirements of the relevant regulations and reflects all sections of the dissertation. It is in a volume of 32 pages, impressing the very good technical layout and the balanced content of its main sections.

11. Critical remarks and recommendations

I have no comments, recommendations or questions.

12. Personal impressions

Since I do not know the PhD student Tsvetelina Georgieva Andonova, I cannot give my personal impressions.

13. Recommendations for future use of dissertation contributions and results

Considering the pioneering nature for our country of research on the phytochemical composition and biological activities of invasive alien plant species, as well as the interest that the published results arouse among the international scientific community, I recommend continuing research in this direction with the inclusion of other species of this group.

CONCLUSION

The dissertation **contains scientific and scientific-applied results, which represent an original contribution to science and meet all the requirements** of the Law 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 Paisiy Hilendarski University of Plovdiv. 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 Paisiy Hilendarski University of Plovdiv for the application of ZRASRB.

The dissertation work shows that the doctoral student Tsvetelina Georgieva Andonova **possesses** in-depth theoretical knowledge and professional skills in the scientific specialty "Botany", **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 work, abstract, achieved results and contributions, and ***I propose to the honorable scientific jury to award the educational and scientific degree "doctor" to Tsvetelina Georgieva Andonova*** in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, Doctoral Program Botany.

28.11.2022

Reviewer:

(Prof. Dimcho Zahariev, PhD)