

REVIEW

by

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External member of the Scientific jury for Medical University-Plovdiv (with Order No - 2121/23.12.2024 of the Vice Rector of SRA of the Medical University-Plovdiv) of a dissertation for awarding an educational and scientific degree 'doctor', professional direction 7.3. Pharmacy, PhD Programme "Pharmaceutical Chemistry"

Author: Velislava Todorova, MPharm

Form of PhD study: full-time

Department: Pharmacognosy and Pharmaceutical Chemistry

Title: Pharmacanalytical control of substances with adaptogenic properties from *Rhaponticum carthamoides* Willd.

Scientific supervisor: Assoc. Prof. Kalin Valentinov Ivanov, PhD, Medical University - Plovdiv

1. General presentation of the procedure and the PhD student.

The set of materials is in accordance with Art. 70 (1) of Section I Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and includes the following documents:

- Application to the Rector of MU-Plovdiv for disclosure of the procedure for the defence of a dissertation
- Curriculum Vitae in European format with the PhD student's signature
- a notarised copy of the higher education diploma
- orders for enrolment in doctoral studies, interruption of studies (due to maternity), and continuation of studies; for deduction with right of defence
- order for examining the individual plan and the corresponding protocol for a passing examination or doctoral minimum in the speciality
- minutes of the departmental council for the preliminary discussion of the predissertation work and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury
- dissertation work
- abstract

- list of scientific publications on the dissertation.
- copies of scientific publications
- list of scientific forum participants
- list of citations noticed
- The authenticity of the attached documents.
- other documents related to the procedure.

The PhD student applied three publications. All documents submitted by the PhD student are in full compliance with the Regulations for the terms and conditions for acquiring scientific degrees and holding academic positions in the Republic of Bulgaria, as well as in accordance with the procedure for acquiring the title of Doctor of Medicine at the Medical University of Plovdiv.

2. Brief biographical data of the PhD student

Velislava Dimitrova Todorova graduated with a Master's degree in Pharmacy at the Medical University in Plovdiv in 2020; From 2020 until now, works as an assistant professor in Pharmaceutical Chemistry and Pharmaceutical Analysis in the Department of "Pharmacognosy and Pharmaceutical Chemistry" of the Faculty of Pharmacy at the Medical University - Plovdiv; From 2021, is a full-time PhD student at the same department; In May 2024 acquired the speciality "Analysis of medicinal products". For 2024, she is the winner of the Pythagoras Award for young scientists in the fields of life sciences and medicine.

By Order of the Rector of MU-Plovdiv, dated R-2368/17.12.2021, Velislava Todorova was enrolled in full-time PhD study at the Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv. By Order R-1574/29.11.2024, she was granted the right to defend her dissertation.

3. Actuality of the topic and appropriateness of the goals and tasks

In recent decades, adaptogens have garnered significant interest in modern science due to their ability to normalise bodily functions and enhance resilience under stress. Despite the ongoing synthesis of new molecules, the interest in compounds from natural sources remains exceptionally high, as many of them have demonstrated considerable therapeutic potential. Modern trends in the search for opportunities to modulate processes such as ageing, influencing obesity, and overweight define the subject of the current dissertation, namely the pharmacokinetic control of substances with adaptogenic properties from *Rhaponticum carthamoides* Willd. Adaptogens could be considered as potential agents to modulate these processes because of their ability to improve stress resistance and maintain homeostasis. Among the first classified adaptogens was *Rhaponticum carthamoides* (leuzea), a plant that is endemic to the Siberian region and has deep traditions in folk medicine. Leuzea contains ecdysteroids with various biological effects, such as stimulation of protein synthesis,

improvement of protective properties, and physical endurance. Historically, research on leuzea began in the 20th century in Russia; however, interest in its therapeutic potential remains strong today, particularly in relation to processes such as ageing, obesity, and overweight, as research on adaptogens in these areas is still limited.

In this regard, the present dissertation proposes strategies to analyse and evaluate the potential of *Rhaponticum carthamoides* in these areas, which represent a significant challenge nowadays and undoubtedly underscore the relevance of the topic.

4. Knowing the problem

PhD student Velislava Todorova has prepared a detailed systematic review of the modern scientific literature related to adaptogens, ecdysteroids, and analytical approaches to their research, demonstrating a thorough understanding of the subject. She presented a comprehensive 49-page literature review, structured into four chapters. The first chapter describes the botanical characteristics and phytochemical composition of key plants with adaptogenic properties, providing a detailed discussion of their biological effects. The second and third chapters focus on the main phytoecdysteroids, such as 20-hydroxyecdysone, turkesterone, and ponasterone A. These chapters emphasize their chemical structure, biological properties, effects on the body, and the specific plants that contain 20-hydroxyecdysone. The final chapter presents the current analytical methods for studying ecdysteroids, including thin-layer chromatography, high-performance liquid chromatography, and gas chromatography.

The literature review is logically organised and offers a comprehensive overview of modern scientific achievements related to the study of adaptogens and their therapeutic potential, thereby demonstrating good theoretical preparation and knowledge of the problem by a PhD student. Current trends and challenges in research on *Rhaponticum carthamoides* and similar plants are carefully analysed and thoroughly evaluated, making them the basis of this dissertation.

5. Research methodology

The methods chosen for the study of *Rhaponticum carthamoides* and its bioactive substances seem appropriate and compatible with the goals set for this dissertation. They cover various approaches, from morphological analysis to high-tech analytical methods and biological tests, which guarantees the complexity and reliability of the results. Methods have been developed and validated to characterise the extracted essential oils of *Rhaponticum carthamoides* (wild and cultivated population) by high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography with a UV-Vis detector (HPLC). To achieve better analysis quality and higher efficiency, the present HPLC method was further developed and adapted for use with LC-PDA-MS,

while an additional LC-MS method was developed and validated. Evaluation and analysis of the anti-adipogenic properties of *Rhaponticum carthamoides*, 20-HE, PA, and TS extracts in an *in vitro* model with human adipocytes. Furthermore, a methodology was applied to evaluate the influence of *Rhaponticum carthamoides* and 20-HE on the ageing processes and lifespan of *C. elegans*.

The methodologies for each technique are described in detail and with great precision. The PhD student has mastered a variety of specialized techniques, which are effectively applied, ensuring the reliability and high quality of the obtained results.

6. Characterisation and evaluation of the dissertation work

The present dissertation is designed according to the requirements for a dissertation at the Medical University of Plovdiv. The work contains 178 pages, and the data are illustrated with 24 tables and 39 figures. A total of 387 literature sources are cited, 170 of which are in the last 10 years and 114 in the last 5 years. The EMA and WADA websites are also cited with up-to-date information. The data are presented in 24 tables and 39 figures. The dissertation includes the following chapters: Contents - 3 pages, Abbreviations used - 2 pages, Introduction - 2 pages, Literature review - 49 pages, Objective and Tasks - 1 page, Materials and Methods - 16 pages, Results and Discussion – 58 pages, Conclusion – 2 pages, Conclusions – 1 page, Contributions – 1 page, List of publications related to dissertation work - 2 pages, References - 38 pages.

The structure of the different chapters is well-organized and logically arranged. The aim of the dissertation is clearly stated, and seven tasks are defined to achieve this goal. The materials and methods used are described in detail. The results and discussion form the main part of the work, presented with high precision and accuracy. The experimental results follow the sequence of the defined tasks and are organized into the following directions:

- A structural evaluation of the plant materials was conducted through microscopic observations to determine the histolocalization of secretory structures in the roots and rhizomes of both wild and cultivated *Rhaponticum carthamoides*.
- The gas chromatographic analysis (GC-MS) of the extracted essential oil from the two populations of wild and cultivated *Rhaponticum carthamoides* revealed a difference in their chemical composition.
- The developed high-performance thin-layer chromatography (HPTLC) method for analyzing *Rhaponticum carthamoides* plant extracts is an effective approach for both qualitative and quantitative determination of plant components. This technique

allows for a detailed study of the chemical composition of plant extracts, providing accurate and reliable results, validated against international standards.

- The developed method for high-performance liquid chromatography with UV-VIS detection (HPLC) provides an opportunity for a reliable analysis of plant extracts of *Rhaponticum carthamoides* and other pharmacologically active compounds, such as 20-HE, PA, and TS. The procedure is optimised by using a gradient mode of operation concerning the mobile phase, which increases the resolution and detection of individual components in the sample.
- The developed LC-MS method for the quantitative determination of 20-XE, PS and TS in plant extracts has high precision, accuracy and sensitivity. The method is resistant and stable when storing samples of the plant extracts, making it suitable for scientific studies of the indicated components.
- The study of the anti-adipogenic potential of *R. carthamoides* extract, 20-HE, PA, and TS in an *in vitro* model of human adipocytes was based on evaluation of its effects on adipogenesis and lipolysis. Based on the use of cell cultures and various biological tests, these compounds were screened for their activity in terms of reducing lipid accumulation and stimulating lipolysis. These studies may serve as a basis for developing new natural remedies to combat obesity and metabolic disorders.
- This study evaluated the effects of *Rhaponticum carthamoides* extract and 20-hydroxyecdysone on vitality and ageing in the *C. elegans* model. Motility, chemosensory activity, and thermal stress resistance tests were performed, which showed significant improvements in the nematodes used. The results highlight the anti-ageing properties of these natural compounds, demonstrating an increase in survival and response to stressors. The findings of this study contribute to our understanding of the biological mechanisms of ageing and demonstrate the potential for application in therapies to prolong life and improve health.

The results of the experimental tasks led to seven conclusions, which are accurate, logical, and effectively summarize the full range of data obtained. Additionally, eight contributions are listed.

7. Contributions and significance of the PhD thesis for science and practice

The dissertation presented by the PhD student includes eight contributions, which can be divided into three with scientific-theoretical value and five with scientific-applied significance. The

contributions of scientific and theoretical value include the first histochemical localization of secretory channels and lipid accumulation in the roots and rhizomes of *Rhaponticum carthamoides*. For the first time, essential oil from *R. carthamoides* cultivated in Bulgaria was extracted and characterized, and the quantitative content of 20-HE, TS, and PA was also determined for the first time.

Particular attention should be given to the contributions of scientific and applied nature. Rapid and highly sensitive analytical methods have been developed for the identification and analysis of 20-HE, TS, and PA, namely the HPTLC method for determining 20-HE, TS, and PA in plant extracts, the HPLC-UV method for analysing 20-HE, TS, and PA in plant extracts and in dietary supplements and LC-PDA-MS method for analysis of 20-HE, TS, and PA. The anti-adipogenic activity of *R. carthamoides* extract and 20-HE was observed in SGBS cells. A positive influence of *R. carthamoides* on aging and lifespan was observed in *C. elegans*.

8. Evaluation of publications related to PhD thesis

In connection with the procedure for the defence of her dissertation, Velislava Todorova presents 3 publications with IF, with a total of 58 citations without self-citations. The PhD student is the first author of all the presented publications. She participated in 4 international conferences, where she presented part of the scientific results of her dissertation. He is a leading researcher on 1 scientific project.

9. Personal participation of the PhD student

I believe that the contributions of this dissertation are largely the work of the PhD student, which was carried out under the competent guidance of her scientific supervisor, Assoc. Prof. Kalin Valentinov Ivanov, PhD. The personal contribution of Velislava Todorova is confirmed by the fact that she is the first author in all presented publications and in three of the scientific forums participation.

10. Summary of the PhD thesis

The abstract complies fully with the requirements laid down in the regulations of the Medical University of Plovdiv. The results are presented briefly and clearly. The abstract is presented in 53 pages, including 11 tables and 19 figures. The content provides a clear understanding of the set goals, the tasks developed, the results obtained, their discussion, and the conclusions and contributions of the dissertation.

11. Critical remarks and recommendations

The PhD thesis is written in excellent scientific language, with a clear structure, thoroughness, and an engaging approach. Given the high value of the research conducted and the materials used, I have no critical remarks or recommendations regarding this dissertation.

12. Personal impressions

I do not know the PhD student Velislava Todorova personally, but after reviewing her biographical data, I was deeply impressed to learn that she is a recipient of the Pythagoras Award for Contribution to Science, awarded to young scientists in the field of life sciences and medicine in 2024. This accomplishment is an indisputable testament to her high professional qualities and skills.

13. Recommendations for future use of the dissertation and results

The contributions and results of this dissertation open many opportunities for future research and applications across various scientific fields. Continued investigation into the anti-adipogenic activity of *R. carthamoides* extract and 20-HE provides a foundation for developing innovative therapeutic approaches. The analytical methods developed can also be applied to other plants containing similar chemical compounds, thereby expanding the database for such plants. Furthermore, improving methods to study lipid metabolism is crucial for understanding the mechanisms underlying metabolic diseases.

CONCLUSION

The dissertation contains scientific, applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB, and the relevant Regulations of the MU- Plovdiv. The materials and results of this dissertation fully correspond to the specific requirements of MU - Plovdiv.

The dissertation demonstrates that the PhD student Velislava Dimitrova Todorova possesses in-depth theoretical knowledge and professional skills in the scientific speciality "Pharmaceutical Chemistry" by demonstrating qualities and skills for independent conduct of scientific research.

Based on the above, I confidently give my positive assessment of the research presented in the dissertation, abstract, achieved results, and contributions. I propose to the esteemed scientific jury to award the educational and scientific degree of "Doctor" to Velislava Dimitrova Todorova, a PhD candidate in the program of "Pharmaceutical Chemistry."

06.01.2025

**Reviewer: Assoc. Prof. Sylvia
Yordanova Atanasova-Stamova, PhD**

Заличено на основание
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