

REVIEW

by

Prof. Svetlana Fotkova Georgieva, PhD

Dean of the Faculty of Pharmacy, member of the Department of "Pharmaceutical Chemistry", Faculty of Pharmacy at the Medical University - Varna "Prof. Dr. Paraskev Stoyanov"

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

Author: Velislava Todorova, MPharm

Form of PhD study: full-time

Department: "Pharmacognosy and Pharmaceutical Chemistry"

Title: "Pharmacoanalytical 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 presented set of materials is under 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 work
- curriculum vitae in the European format with the PhD student's signature
- a notarised copy of a 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 conducting an exam from the individual plan and the corresponding protocol for a passed exam or doctoral minimum in the speciality
- minutes of the departmental council for the preliminary discussion of the pre-dissertation work and the decisions taken for the disclosure of the procedure and for the composition of the scientific jury

- dissertation work
- abstract of the dissertation
- list of scientific publications on the topic of the dissertation
- copies of the scientific publications
- list of participations in scientific forums
- list of citations noticed
- declaration of originality and authenticity of the attached documents
- other documents related to the course of the procedure

The PhD student has attached 3 publications with a total impact factor of 14.306.

2. Brief biographical data of the PhD student

Velislava Dimitrova Todorova earned her Master's degree in Pharmacy from the Medical University of Plovdiv in 2020. From 2020 until now, she has worked as an assistant 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". The PhD student is a winner of the "Pythagoras" award for a young scientist in the field of life sciences and medicine – 2024.

3. Actuality of the topic and appropriateness of set objectives and tasks

Adaptogens are a subject of considerable interest in the modern scientific community because of their ability to normalise physiological processes in the body and strengthen it under stress conditions. *Rhaponticum carthamoides* (leuzea) is a leading source of active compounds with a pronounced adaptogenic effect. It is believed that the adaptogenic effects of leuzea are related to the presence of ecdysteroids (ecdysterone, turkesterone, ponasterone, and others) - a group of natural compounds with diverse biological effects. Ecdysteroids have many pharmacological properties, including stimulation of protein synthesis, protective effects, and increased physical endurance. Despite advances in the synthesis of new molecules, there remains considerable interest in plant ecdysteroids due to their significant therapeutic potential.

Ecdysteroids are characterised by a diverse range of structural polymorphisms. Structural differences in the number of carbon atoms in the side chains and substituents at certain positions lead to the formation of compounds with different polarities, which require the use of various analytical methods for their isolation and identification.

Despite the availability of existing techniques for the analysis of ecdysteroids, there remains a need to develop new analytical approaches that provide higher sensitivity, speed, and reliability.

Chromatographic methods are among the most widely used techniques for the analysis of ecdysteroids: thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and supercritical fluid chromatography (SFC) as well as nuclear magnetic resonance. In addition, biological methods, such as radioimmunological analysis, are also used. Current methods often require complex sample preparation or long analysis times, making them unsuitable for routine use. The development of simpler and more environmentally friendly methods will facilitate qualitative and quantitative determination of ecdysteroids in plant extracts.

The relevance of the topic is driven by the need to develop, optimise, and validate new analytical methods for the control and analysis of compounds with adaptogenic properties extracted from *Rhaponticum carthamoides*, as well as to assess their potential biological activity. The results of the conducted research lay the groundwork for future studies on the molecular mechanisms of action of adaptogens from *Rhaponticum carthamoides* and their potential application in improving the quality of life, highlighting the contemporary significance and relevance of the dissertation work of PhD student Velislava Dimitrova Todorova.

4. Understanding the problem

The literature review is written in an excellent academic style. Velislava Todorova shows excellent knowledge of scientific information on the issue. Theoretical and practical knowledge and skills for working with HPTLC, HPLC-UV, and LC-PDA-MS methods for pharmaceutical analysis are presented, as well as basic biochemical models for evaluating the biological activity of adaptogens from *R. carthamoides*.

5. Research methodology

The chosen research methods enable the achievement of the set objectives and provide an adequate response to the tasks addressed in the dissertation.

6. Characterisation and evaluation of the dissertation work

The dissertation consists of 178 pages and contains 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 the dissertation work – 2 pages, References – 38 pages.

The data are presented in 24 tables and 39 figures. 387 sources of literature 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 technical layout of the dissertation meets all the requirements.

As a result of the review, clear objectives were logically derived: to develop, optimise, and validate new analytical methods for the control of substances with adaptogenic properties extracted from *R. carthamoides* and to assess their potential biological activity.

Seven tasks are set that correspond to the goal and are scientifically based. They were implemented in stages during the development of the dissertation work. In fulfilling the tasks of the dissertation, the effects of *R. carthamoides* extract, 20-hydroxyecdysone, turkesterone, and ponasterone A on adipogenesis in an *in vitro* model of human adipocytes and ageing processes in *C. elegans* were investigated. Extracts of *R. carthamoides* and 20-hydroxyecdysone were found to reduce adipogenesis and stimulate lipolysis, turkesterone only affected adipogenesis and ponasterone A had no such effects. In addition, the extract of *R. carthamoides* affected lipid metabolism in *C. elegans*. The extract of *R. carthamoides* was shown to extend lifespan, delay physiological ageing in *C. elegans*, and enhance resistance to heat stress as well as survival under oxidative stress. In contrast, 20-hydroxyecdysone increased survival in *C. elegans* exposed to oxidative stress and improved their resistance to heat stress.

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

I fully accept the contributions mentioned by the PhD student. **Original contributions** were the histochemical localisation of secretory channels and lipid accumulation in the roots and rhizomes of *R. carthamoides*; the extraction and characterisation of essential oil from *R. carthamoides* cultivated in Bulgaria and the determination of the quantitative content of 20-hydroxyecdysone, turkesterone, and ponasterone A in *R. carthamoides* cultivated in Bulgaria.

Methodological contributions are related to the development of fast and sensitive HPTLC, HPLC-UV, and LC-PDA-MS methods for the pharmaceutical analysis of 20-hydroxyecdysone, turkesterone and ponasterone A in plant extracts.

In terms of scientific contributions, two key findings were formulated: the anti-adipogenic activity of *R. carthamoides* extract and 20-hydroxyecdysone in SGBS cells was established, and a positive effect of *R. carthamoides* on ageing and lifespan in *C. elegans* was observed.

8. Evaluation of publications on dissertation work

In the dissertation, Velislava Todorova has attached 3 articles with IF, with a total of 58 citations without self-citations. The PhD student is the first author of all submitted publications. There have been 4 participations in international conferences and one participation in scientific projects as a leading researcher.

The scientific activity of Velislava Todorova, MPharm exceeds the required minimum criteria for the educational and scientific degree "Doctor" according to the requirements of the Law on the

Development of the Academic Staff in the Republic of Bulgaria and the Regulations of the MU-Plovdiv.

9. Personal participation of the PhD student

From the text of the dissertation, the abstract, and the presented publications, the personal involvement of Velislava Todorova, MPharm, is clearly demonstrated.

10. Abstract of the PhD thesis

The abstract reflects the essence of the dissertation work and meets the requirements. The abstract consists of 53 pages and contains the following chapters: Contents - 1 page, Abbreviations used - 1 page, Introduction - 1 page, Objective and Tasks - 1 page, Materials and Methods - 7 pages, Results and Discussion - 35 pages, Conclusion - 1 page; Conclusions - 1 page, Contributions - 1 page, List of publications related to the dissertation work - 1 page. The data are presented in 11 tables and 19 figures.

11. Critical remarks and recommendations

I have no critical remarks or recommendations regarding the research conducted or the materials presented.

CONCLUSION

I believe that the presented dissertation work of Velislava Dimitrova Todorova, MPharm is up-to-date and was conducted using the most modern analytical research methods and has original, methodological, and scientific-applied contributions to modern pharmaceutical science and practice. The structure, content, and volume meet all the regulatory requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB, and the Regulations of the MU-Plovdiv. The materials presented and the results fully correspond to the specific requirements adopted in connection with these Regulations.

The dissertation shows that the PhD student Velislava Dimitrova Todorova, MPharm possesses in-depth theoretical knowledge and professional skills in the scientific speciality "Pharmaceutical Chemistry", demonstrating qualities and skills for independent 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 honourable scientific jury to award the educational and scientific degree "Doctor" to Velislava Dimitrova Todorova, MPharm in the PhD programme in "Pharmaceutical Chemistry".

06.01 2025

Reviewer:

(Prof. Sv. Заличено на основание
Чл.5 §1, б."В" Регламент (ЕС)2016/679)

