STATEMENT

by Assoc. Prof. Diana Petrova Karcheva-Bahchevanska, PhD

Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University - Plovdiv

regarding a procedure for acquiring the educational and science degree "Doctor", professional field 7.3. Pharmacy,

Doctoral Program "Pharmaceutical Chemistry"

Author: MPharm Velislava Dimitrova Todorova

Form of the doctoral program: Full-time

Department of Pharmacognosy and Pharmaceutical Chemistry

Topic: "Pharmacoanalytical control of substances with adaptogenic properties from *Rhaponticum carthamoides* Willd."

Scientific supervisor. Assoc. Prof. Kalin Valentinov Ivanov, PhD, Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Ploydiv.

1. General presentation of the procedure and the PhD student

The submitted set of paper/electronic materials is in accordance with Article 70 (1) of Section I. Acquisition of Doctoral Degree at MU-Plovdiv; Regulations of MU-Plovdiv of 28.01.2021 and includes all required documents.

Velislava Dimitrova Todorova graduated in Pharmacy, Master's degree at Medical University of Plovdiv in 2020. Since 2020 she has been working as an assistant professor of Pharmaceutical Chemistry and Pharmaceutical Analysis at the Department of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Pharmacy at Medical University of Plovdiv. Since 2021 she has been a full-time PhD student at the same department. In May 2024 she acquired the specialty "Analysis of medicinal products".

In connection with the development of her dissertation, the PhD student has participated in 4 international scientific forums. She is the principal investigator of 1 intra-university research project (DPDP-06/2022). She has submitted 3 (three) publications in journals with an impact factor, with a total number of citations - 58, excluding self-citations. She is the first author of the publications submitted.

2. Topical relevance

The topic of the presented dissertation is scientifically significant. It offers reliable strategies for analytical characterization as well as approaches to evaluate the therapeutic potential of *Rhaponticum carthamoides* Willd. and ecdysteroids in overweight and aging. Modulating processes such as aging and influencing obesity are among the areas that pose a serious challenge in our modern era. The dissertation work integrates research in the fields of pharmaceutical chemistry and analysis, pharmacognosy and phytochemistry, and

pharmacology, making the results presented theoretically and practically relevant to pharmacy and medicine in general.

3. Knowledge of the problem

The literature review covers in detail all aspects of the problem at hand. In shaping the literature review, describing and interpreting the results, 387 literature sources were cited, 170 of which were within the last 10 years and 114 within the last 5 years. EMA and WADA websites with up-to-date information were also cited.

The aim of the study is clearly formulated and is achieved by solving seven well-founded tasks.

Regarding the elucidation of phytochemical composition - histochemical analysis was carried out to determine the presence of secretory structures and lipophilic compounds in roots and rhizome of *R. carthamoides*, cultivated in Bulgaria and wild species in Russia. Essential oil (EO) was isolated from the Bulgarian-cultivated population of *R. carthamoides*, the composition of which was compared by GC-MS with that of the wild population.

The quantitative contents of 20-Hydroxyecdysone (20-HE), Ponasterone A (PS) and Turkesterone (TS) in the prepared extracts of *R. carthamoides* from the two populations were compared using validated HPTLC, HPLC-UV, and LC-PDA-MS methods.

Regarding the biological activity studied - the effects of *R. carthamoides* and the investigated phytosteroids on adipogenesis in an *in vitro* model of human adipocytes and the aging processes in *Caenorhabditis elegans*.

4. Research methodology

In this dissertation, the **Materials and Methods** section describes in detail the methods used to perform the previously set tasks. A prerequisite for acquiring the necessary theoretical knowledge and practical skills is the use of a range of methods - microscopic histochemical analysis, validated HPTLC, HPLC-UV, and LC-PDA-MS methods, *in vitro* anti-adipogenic activity and lifespan in *C. elegans* have been monitored. This gives reason to consider that the dissertation has the necessary scientific value, and the PhD student, in the process of her studies, has acquired the ability for independent future research.

5. Characteristics and evaluation of the thesis and contributions

The dissertation submitted by Velislava Todorova fully meets the requirements of MU-Plovdiv for the award of PhD.

The dissertation is organized in 178 pages and contains the necessary main sections (Content - 3 pages, Abbreviations - 2 pages, Introduction - 2 pages, Literature review - 49 pages, Aim and objectives - 1 page, Materials and methods - 16 pages, Results and discussion - 58 pages, Conclusions - 3 page, Contributions - 1 page, List of publications related to the dissertation - 2 pages, References - 38 pages).

The data are presented in 24 tables and illustrated with 39 figures. The bibliographic reference includes 387 literature sources, 114 of them in the last 5 years, which confirms the topicality of the subject.

The following contributions are reported:

- ✓ For the first time, histochemical localization of secretory ducts and lipid accumulations in roots and rhizome of *R. Carthamoides* is conducted;
- ✓ For the first time, essential oil of the species cultivated in Bulgaria has been isolated and characterized:
- ✓ Rapid and highly sensitive HPTLC, HPLC-UV, LC-PDA-MS methods have been established for the analysis of 20-HE, TS and PS in plant extracts and dietary supplements;
- ✓ For the first time, the content of 20-HE, TS and PS in *R. carthamoides* cultivated in Bulgaria was quantified;
- ✓ The anti-adipogenic activity of *R. carthamoides* and 20-HE in SGBS cells was evaluated;
- ✓ A positive influence of *R. carthamoides* on aging and lifespan in *C. elegans* has been verified.

6. Assessment of publications and personal contribution of the PhD student

The presented list of publications related to the dissertation contains 3 scientific publications in journals with an impact factor. The doctoral candidate is the first author of each of them. The results of this dissertation have also been promoted through participation in 4 international scientific forums. Velislava Todorova is the lead researcher in one intra-university research project.

I believe that the research carried out has the **necessary personal contribution** of the PhD student for this kind of work.

7. Abstract

The presented abstract contains 53 pages and is structured correctly as it fully reflects the studies conducted, results obtained and contributions made in the thesis. The abstract thus formatted fully meets the requirements under the Regulations of MU-Plovdiv for the acquisition of educational and scientific degree "Doctor".

CONCLUSION

The PhD student Velislava Todorova meets the requirements for obtaining the PhD degree at the Medical University - Plovdiv. Her dissertation thesis entitled "Pharmacoanalytical control of substances with adaptogenic properties from *Rhaponticum carthamoides* Willd." contains significant scientific, scientific-applied and applied results, which represent an original contribution to the field of pharmaceutical chemistry and analysis. The submitted work fully complies with all the requirements of the Academic Staff Development Act in the Republic of Bulgaria and complies with the specific requirements specified in the regulations for academic development at the Medical University - Plovdiv.

The PhD student Velislava Todorova possesses an in-depth theoretical knowledge of the scientific specialty "Pharmaceutical Chemistry", demonstrating skills for independent scientific research.

Due to the above, I confidently give my positive assessment for the conducted research, achieved results and contributions, and I propose to the honorable scientific jury to award the

educational and scientific degree "Doctor" to Velislava Dimitrova Todorova in the PhD program "Pharmaceutical Chemistry", in the field of higher education 7. Health and Sport, professional field 7.3. Pharmacy.

10.01.2025 г.