OPINION

by Assoc. Prof. Maya Petrova Radeva-Ilieva, MScPharm, PhD

Head of Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna

regarding

procedure for defending a dissertation work for the acquisition of an educational and scientific degree **"Doctor"** in the field of higher education 7. Healthcare and sport, professional field 7.3. Pharmacy, doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy)"

of

Yoana Nikolova Sotirova – full-time doctoral student at the Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy at MU-Varna

on the topic

"Nanostructured lipid carriers loaded with *Hypericum perforatum L.* extract for dermal application and accelerated wound healing"

scientific supervisors

Prof. Kaloyan Dobrinov Georgiev, MScPharm, PhD, DSc

Assoc. Prof. Velichka Yordanova Andonova, MScPharm, PhD

On the basis of Order No. P-109-476/18.12.2024 of the Rector of Medical University – Varna I was elected as a member of the Scientific Jury, and on the basis of Protocol No. 1/30.12.2024, I was appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Doctor" by Yoana Nikolova Sotirova.

The present opinion is prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), as well as the Regulations for the Implementation of the LDASRB (RILDASRB) and the Regulations for the Development of the Academic Staff (RDAS) at MU – Varna. The documents submitted by the candidate meet the specified requirements and are prepared extremely precisely.

I declare that I have no joint scientific works with the doctoral student.

Biographical data of the candidate

Yoana Nikolova Sotirova was born in 1996 in Yambol. In 2015, she graduated from the Mathematical High School "Atanas Radev" in Yambol and in the same year she started to study pharmacy at Medical University "Prof. Dr. Paraskev Stoyanov", Varna. In 2019, she was awarded the annual award of the Bulgarian Pharmaceutical Union for pharmacy students in the Faculty of Pharmacy. In 2020, she graduated with honors at the Faculty of Pharmacy of MU-Varna and acquires the qualification "Master of Pharmacy" and became the winner of the "Golden Galen" award. Since February 2021, she has been appointed as an assistant professor at the Department of Pharmaceutical Technologies at the Faculty of Pharmacy in Medical University "Prof. Dr. Paraskev Stoyanov" - Varna. At the end of 2021, she was enrolled as a full-time doctoral student in the doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy)" at the Department of "Pharmacology, Toxicology and Pharmacotherapy" of the Faculty of Pharmacy at MU-Varna. In 2024, she acquired a specialty in "Drug Technology with Biopharmacy".

Relevance of the dissertation topic

Nowadays, despite the rapid development of medicine and pharmacy, wound healing continues to be a serious problem worldwide. This is partly related to the fact that wound healing is a complex and dynamic physiological process, which is influenced by a number of exogenous and endogenous factors. The currently used therapeutic agents and approaches, however, do not always achieve the desired success. This necessitates the search and introduction of new medicinal substances with improved activity and broad-spectrum action. At the same time, a number of medicinal plants have shown beneficial effects in the treatment of wounds by exerting anti-inflammatory, antimicrobial and epithelializing effects. For example, St. John's wort (*Hypericum perforatum L.*) is a medicinal plant that is used in some mental illnesses (depression, anxiety and insomnia), as well as in the therapy of skin wounds of various origins. The pharmacological effects of St. John's wort are believed to be mainly due to the biologically active substance hyperforin, which is mainly found in the flowers and leaves of the plant. However, hyperforin is a compound with extremely low chemical stability, which significantly limits its use and poses a serious challenge for its inclusion in dosage forms for dermal application.

Based on the above, I believe that the present dissertation paper addresses a current and significant topic related to the possibility of developing an innovative dosage form containing a hyperforin-rich St. John's wort extract, intended for dermal application for the purpose of accelerated wound healing.

Structure of the dissertation work

The dissertation work of Yoana Sotirova is very well structured and has been prepared in accordance with the requirements for obtaining the educational and scientific degree "Doctor". The dissertation work contains a total of 123 pages, divided into the following mandatory sections:

I. Introduction – 1 p.;
II. Literature review – 36 p.;
III. Aim and tasks – 2 p.;
IV. Materials and methods – 13 p.;
V. Results and discussion – 34 p.;
VI. Conclusions – 2 p.;
VII. Scientific contributions – 1 p.;
VIII. Bibliography – 27 p.;
IX. List of scientific publications related to the dissertation work – 1 p.

The dissertation is illustrated with 38 figures and 17 tables. The literary sources used are a total of 387, of which only 1 is in Bulgarian.

The literature review is systematically presented and contains a sufficient volume of summarized and analyzed scientific material. It is divided into several parts, which sequentially examine: the anatomical and physiological features of the skin; wounds as a pathological skin problem and the stages of wound healing; the phytochemical composition and use of St. John's wort as a medicinal plant; nanoscale drug delivery systems, methods for their preparation, advantages and disadvantages, lipid nanoparticles as carriers of biologically active substances are described and characterized in detail; semi-solid dosage forms for dermal application, the technology for their preparation, advantages and disadvantages are examined. At the end of the section, the doctoral student presented the conclusions from the prepared literature review, which are directly related to the formulated goal and show a very good knowledge of the subject.

The aim and tasks of the present dissertation work are logically related to the prepared literature review. **The aim** of the dissertation is precisely and clearly formulated and is focused on the development of nanostructured lipid carriers with St. John's wort extract, intended to be applied dermally for accelerated wound healing. **The tasks** are 4 in total and arise from the set purpose. They are precisely formulated and justified, and their implementation enables the achievement of the defined purpose.

The materials and methods used are appropriately selected with regard to the tasks set and their implementation. Numerous studies have been conducted for the realization of the dissertation work: preparation of St. John's wort extract and subsequent liquid chromatographic analysis for its

qualitative and quantitative characterization, preparation and characterization of nanostructured lipid carriers, preparation and characterization of bigels, *in vivo* study of pharmacological activity by studying the wound healing potential of the prepared final semi-solid dosage form in experimental animals and statistical analysis of the obtained results. It's impressive that a wide variety of methods in different scientific fields are used, which shows the high scientific value of the present dissertation work. The experimental work performed is presented in detail.

The results and discussion are combined into one section. They are well structured and follow the tasks set, with each presented result followed by a discussion on its significance and interpretation. It is very impressive that every statement or assumption is supported and substantiated with scientific information. The results are accompanied by numerous figures and tables. I believe that the results obtained are original and reliable, supported by a large volume of analyzed and summarized scientific material. The strict consistency shown in the doctoral student's work is impressive - from establishing the influence of experimental conditions on the stability and physicochemical characteristics of the proposed nanolipid carriers, which resulted in the selection and subsequent characterization of two optimal compositions of nanocarriers for St. John's wort extract, to the development and characterization of bigels with different compositions, which lead to the selection of optimal hydrogel-oleogel ratio for the preparation of bigel loaded with St. John's wort extract for *in vivo* application to establish its antioxidant and wound healing effect.

As a result of the research conducted, specific **conclusions** have been formulated that have a practical focus and are in accordance with the collected and analyzed data. I believe that all conclusions drawn objectively reflect the results obtained by the doctoral student.

The scientific contributions of the dissertation work are well formulated and are divided into those of a scientific-theoretical and scientific-applied nature.

Summary of the dissertation work

The summary of the dissertation work has been prepared according to the requirements and contains a total of 48 pages. It is structured correctly and presents important figures and tables.

The candidate's scientific activity related to the dissertation work

Two publications in scientific journals are presented, in which the doctoral student is the first author. One of the articles was published in an international journal with IF, which is indisputable evidence of the high scientific value of the research conducted and the results obtained. The doctoral student presents three participations in scientific conferences related to the topic of the dissertation work – 2 in national forums and 1 in an international one (Malmö, Sweden). In addition, the doctoral student participates in two scientific research projects.

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CONCLUSION

The dissertation work of Yoana Nikolova Sotirova on the topic " Nanostructured lipid carriers loaded with *Hypericum perforatum L.* extract for dermal application and accelerated wound healing " fully complies with the requirements of LDASRB, RILDASRB and RDAS of MU-Varna. The dissertation work is dedicated to a current topic. Many and diverse methods were used for the experimental work that lead to obtaining of significant results, which represent an original contribution to pharmaceutical science and practice.

The present dissertation work demonstrates that Yoana Sotirova possesses in-depth theoretical knowledge, has mastered a wide range of experimental methodologies and has gained significant practical experience. The doctoral student demonstrates qualities and abilities for independent research, which is an excellent prerequisite for its successful future development as a scientist.

Based on the above, I give my **POSITIVE ASSESSMENT** of the present dissertation work and propose to the esteemed Scientific Jury to award the educational and scientific degree "**Doctor**" in the field of higher education 7. Health and Sport, professional field 7.3. Pharmacy, doctoral program "Pharmacology (incl. pharmacokinetics and chemotherapy)" to **YOANA NIKOLOVA SOTIROVA**.

27.01.2025
Varna

Prepared by: .....
(Assoc. Prof. Maya Radeva-Ilieva, PhD)