

OPINION

by **Assoc. Prof. Silvia Gancheva Marinova, MD, PhD**

Head of the Department of Pharmacology and Clinical Pharmacology and Therapeutics at the Faculty of Medicine at Medical University "Prof. Dr. Paraskev Stoyanov"

regarding

a dissertation for acquisition of educational and scientific degree "**Philosophy Doctor**" in Higher Education Area 7. Healthcare and Sport, Professional Field 7.3. Pharmacy, Doctoral Programme „Pharmacology (incl. Pharmacokinetics and Chemotherapy)" of

Yoana Nikolova Sotirova – a doctoral student in full-time training at the Department of Pharmacology, Toxicology and Pharmacotherapy at Medical University of Varna

on the topic „**Nanostructured Lipid Carriers Loaded with *Hypericum perforatum* L. Extract for Dermal Application and Accelerated Wound Healing**"

Research supervisors: Prof. Kaloyan Georgiev, PhD, DSc and Assoc. Prof. Velichka Andonova, PhD

On the basis of Order № P-109-476/18.12.2024 of the Rector of Medical University of Varna and decision of the Scientific jury (Protocol № 1), I am appointed to prepare an opinion on the procedure for acquiring the educational and scientific degree "Philosophy Doctor" to Yoana Nikolova Sotirova.

The submitted materials for the procedure meet the requirements of the Law on Development of Academic Staff in Republic of Bulgaria and the Regulations for its application at Medical University of Varna.

Biographical data

Yoana Sotirova was born in 1996. She completed her secondary education in 2015 at "Atanas Radev" High School, Yambol. She graduated as a "Master" in pharmacy in 2020 at the Medical University of Varna with full honors and the "Golden Galen" award. While she was a student, she worked as a trainee pharmacist in pharmacies in Yambol and Varna. After graduation, she was briefly a master pharmacist at the pharmacy "St. Luka - 2", Varna. In February 2021, after winning a competition, she was appointed to the position of a full-time assistant professor at the Department of Pharmaceutical Technologies at Pharmacy Faculty of Varna Medical University. She acquired a specialty "Drug Technology with Biopharmacy" in 2024. Yoana Sotirova holds a certificate of good clinical practice from the Bulgarian Association of Clinical Research. Yoana Sotirova is a participant in 2 research projects and a co-author of 13 scientific publications, 8 of which are published in high impact journals. She has participated in 13 scientific forums, and her research has been awarded 2 times with prizes for poster presentation. She speaks English fluently and has excellent computer skills and digital competences. Yoana Sotirova is a member of Bulgarian Pharmaceutical Union.

Relevance of the topic

The development of science and technology makes possible the confirmation of empirical knowledge of the beneficial properties of medicinal plants in various health problems by clarifying their active principles and mechanisms of action. Since plant extracts and biologically active substances of natural origin evoke greater trust among the patients than the synthetic drugs and demonstrate a good safety profile, the scientific interest in this field has been growing in recent years.

One of the health problems that are favorably affected by medicinal plants is wound healing. The process is often complicated, usually as a result of microbial colonization and oxidative stress, leading to an extension of the inflammatory phase and chronification. Many plant extracts and biologically active substances of natural origin exhibit anti-inflammatory, antioxidant and antibacterial effects, which can explain their beneficial effect on wound healing.

St. John's wort (*Hypericum perforatum* L.) is one of the most popular medicinal plants worldwide. Although it is usually used for its antidepressant effect, St. John's wort and its biologically active substances also have a pronounced wound-healing potential, as they stimulate the processes of collagen formation, keratinocyte differentiation and re-epithelialization in addition to their antioxidant and anti-inflammatory properties. The main drawback of the plant, limiting its use in wound healing, is the low structural stability of its main biologically active substance. In addition, the dermal use of plant products in wound healing is often hampered by the low penetration ability of the dosage forms, especially in case of infection and formation of microbial biofilm on the wound bed.

Yoana Sotirova's dissertation is focused on this relevant problem - the necessity for creation of innovative drug delivery systems which improve the stability of the biologically active substances of St. John's wort and provide good penetration into the skin when applied as a dosage form with optimal physico-chemical characteristics.

Structure of the dissertation

Yoana Sotirova's thesis is presented by 123 standard pages. It is designed in accordance with the requirements for acquiring the educational and scientific degree "Philosophy Doctor". The dissertation includes all obligatory sections, which are properly balanced, as follows: Introduction – 1 page, Literature Review – 36 pages, Aim and Objectives – 2 pages, Materials and Methods – 13 pages, Results and Discussion – 34 pages, Conclusions – 2 pages, Contributions – 1 page, References – 27 pages, List of scientific publications and participations related to the thesis – 1 page. The dissertation is illustrated by 38 figures and 17 tables. The bibliography includes 387 references.

The **literature review** is focused on the main issues addressed in the thesis – wounds and wound healing, characteristics of St. John's wort, the features of nano-sized drug delivery systems and semisolid dosage forms for dermal application. The author pays the greatest attention to the phytochemical composition of St. John's wort and to the characteristics of different types of nano-sized drug delivery systems and the methods of their preparation, which is logically linked to the

aim and objectives of the PhD thesis. The literature review is comprehensive and presented clearly and logically. It demonstrates Yoana Sotirova's in-depth theoretical knowledge of the issues covered in the thesis. The review is very well illustrated with 16 figures. I am particularly impressed by the author's figures made with the scientific image and illustration software BioRender.

The **aim and objectives** of the dissertation are logically related to the presented literature review. The aim is precisely and clearly formulated, and the research tasks are adequate for its implementation. I highly appreciate the inclusion of an *in vivo* study in a model of excision wound in experimental animals.

The **materials and methods** used in the dissertation are suitable for the fulfillment of the tasks set. The author presents comprehensively all of the reagents, laboratory and medical supplies, and analytical and general laboratory equipment used. The methods utilized in the study, incl. all of their steps, are described in detail. For the realization of the dissertation, a wide range of research methods were used. They correspond to the tasks and are reported in the same sequence: method for preparation of hyperforin-rich St. John's wort extracts and for qualitative and quantitative analysis of their hyperforin content; methods for preparation and characterization of nanostructured lipid carriers; methods for preparation and characterization of bigels; methods for *in vivo* study in an experimental model of excision wound and for evaluation of antioxidant status of the animals. The *in vivo* study design is appropriately chosen and clearly explained. The statistical analysis is also selected and performed adequately.

The **results and discussion** are combined in one section and follow the tasks. Two St. John's wort extracts were obtained, both of which were found to contain a high concentration of hyperforin. The reasons for choosing one of the extracts for the subsequent experimental work are explained. In order to select the most optimal nanostructured lipid carrier for inclusion of St. John's wort extract, an impressively large number of samples – 20 – were designed. After analyses of their characteristics, 2 samples were determined as optimal for loading with the selected extract. Both of the selected nanostructured lipid carriers generally retained their structure and stability after incorporation of the extract. However, after conducting tests for microbicidal effect and antiviral activity, one of them was chosen for further experimental work. Development of biphasic gels followed – 4 “blank” bigels with different percentage of oil phase and 4 corresponding bigels loaded with the nanostructured lipid carrier enriched with St. John's wort extract. After analyses of their physical characteristics, one of them was selected for conduction of the *in vivo* experiment. The wound-healing effect and antioxidant properties of the bigel containing a nanostructured lipid carrier loaded with St. John's wort extract were compared to those of a bigel containing “unprotected” St. John's wort extract and a commercial herbal product with wound-healing properties used as a positive control. The *in vivo* study confirmed the initial hypothesis that the incorporation of the extract to a nanostructured lipid carrier would improve its beneficial effects on the wound healing process and found that the antioxidant effect of the extract was mainly due to free radicals scavenging. The presentation and discussion of the results is clear and logically justified. The section is very well illustrated by 22 figures and 12 tables. The visualization of the results facilitates their quick perception by the reader.

On the basis of the results, the author forms 7 **conclusions**. They are well formulated and contain the essence of the PhD thesis.

The author formulates 7 **contributions** of the PhD thesis and all of them are original. They are classified in 2 groups – 5 contributions of a scientific and theoretical nature and 2 contributions of a scientific and applied nature. In the dissertation work:

- The optimal lipid composition and experimental conditions for obtaining stable nanostructured lipid carrier were established.
- For the first time, nanostructured lipid carriers were loaded with hyperforin St. John's wort extract.
- An optimal nanostructured lipid carrier of hyperforin-rich St. John's wort extract was formulated.
- For the first time, bigel was used as a semi-solid carrier for the dermal application of nanostructured lipid carriers.
- A bigel with optimal mechanical and rheological characteristics for application to the skin was formulated.
- An original protocol for isolating a St. John's wort extract with a high hyperforin content was developed.
- An innovative semi-solid dosage form, which allows the dermal application of hyperforin-rich St. John's wort extract and provides accelerated wound healing compared to a reference herbal product, was prepared.

Publications and participations in scientific events

Yoana Sotirova presented a list of 2 full-text scientific articles related to the dissertation, in both of which she is the first author. One of them is published in a high impact journal, which proves the scientific value of the dissertation work. The results of the dissertation were presented at 3 scientific forums. The doctoral student was the first author in all of the poster presentations.

Summary of the dissertation

The summary of the dissertation is prepared in accordance with the requirements. The summary consists of 48 pages. It is appropriately structured and illustrated.

Conclusion

The dissertation of Yoana Nikolova Sotirova entitled „Nanostructured Lipid Carriers Loaded with *Hypericum perforatum* L. Extract for Dermal Application and Accelerated Wound Healing” concerns an important health problem and is dedicated to the development of an innovative dosage form. A variety of research methods have been utilized for the implementation of dissertation tasks. Results of high theoretical and practical significance have been obtained, representing an original scientific contribution. The dissertation of Yoana Sotirova meets the requirements of the Law on Development of Academic Staff in Republic of Bulgaria and the Regulations for its application at Medical University of Varna.

The dissertation of Yoana Sotirova shows her ability to use a variety of research methods and competently analyze and present the results obtained. Yoana Sotirova demonstrates in-depth theoretical knowledge and professional skills in scientific field of pharmacology and is capable to conduct independently a scientific research.

In conclusion, I confidently give my **positive** assessment to Yoana Nikolova Sotirova and I offer the esteemed Members of the Scientific Jury to award her the educational and scientific degree “Philosophy Doctor” in Higher Education Area 7. Healthcare and Sport, Professional Field 7.3. Pharmacy, Doctoral Programme „Pharmacology (incl. Pharmacokinetics and Chemotherapy)”

Заличено на основание чл. 5,
§1, б. „В“ от Регламент (ЕС)
2016/679

27.01.2025
Varna, Bulgaria

Prepared by:
/Assoc. Prof. Silvia Gancheva, MD, PhD/

