

**Review for the degree „Doctor“**

**REVIEW**

**by**

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External member of the Scientific jury for Medical University-Plovdiv  
(with Order № P-198/08.10.2024 of the Rector of the Medical University-Plovdiv)

on a of a dissertation for awarding the educational and scientific degree '**doctor**'  
professional field 7.3. "Pharmacy"

doctoral program "Pharmacognosy and Phytochemistry"

**Author: Vanya Ivanova Nalbantova**

**Form of doctoral study:** full-time

**Department:** "Pharmacognosy and Pharmaceutical Chemistry"

**Title:** "Phytochemical investigation and application possibilities of seeds of *Trigonella foenum-graecum* L., spread in our country."

**Scientific supervisors:** Assoc. Prof. Niko Yosif Benbasat, PhD and Prof. Cédric Delattre, PhD

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

The set of materials presented by PhD student 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 dated 28.01.2021 and includes the following documents:

- Application to the Rector of MU-Plovdiv for disclosure of the procedure for the defense of a dissertation work
- CV in European format with the PhD student's signature
- a notarized copy of a higher education diploma
- order for enrollment in doctoral studies
- order for deduction with right of defense
- protocol of a passed exam or doctoral minimum in the specialty
- protocol 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
- a list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- declaration of originality and authenticity of the attached documents

The PhD student has presented four publications. All documents presented 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 “Doctor” at the Medical University - Plovdiv.

## **2. Brief biographical data for the PhD student**

Vanya Ivanova Nalbantova was born on 16.11.1991. In 2016, she completed her higher education at the Faculty of Pharmacy of the Medical University of Plovdiv, obtaining a master's degree in pharmacy. In 2019, Vanya Nalbantova becomes an assistant in the "Pharmacognosy and Pharmaceutical Chemistry" department at the Faculty of Pharmacy of the MU-Plovdiv. In the same Institution, her post-graduate qualification continues, where from 2021 her specialization in "Medicinal Plants and Phytopharmaceutical Products" is in progress. In parallel, Vanya Nalbantova completed an additional qualification course in "Chromatography and mass spectrometry"; as well as several training modules on UV-VIS, FI-IR and Raman Spectroscopy, Gas Chromatography and Gas Chromatography with Mass Spectrometry, Liquid Chromatography, Liquid Chromatography Mass Spectrometry, Ion Chromatography, Fundamentals of High Performance Liquid Chromatography, Fundamentals of Mass Spectrometry, Approaches to Proper Column Selection for Liquid Chromatography, Application of Artificial Intelligence in Liquid Chromatography - The New Shimadzu Nexera LC-40 Series. She has also completed training courses on the operation and maintenance of Infrared Spectrometer (FT-IR) model ALPHA II, Bruker, Portable Turbidimeter model TB1, VELP Scientifica, TitroLine 7800 Combined Potentiometric Titrator and Karl-Fischer Titrator.

By Order of the Rector of MU-Plovdiv dated 17.12.2021, Vanya Nalbantova was enrolled in full-time PhD study at the Department of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Pharmacy of MU-Plovdiv, and on 04.10.2024 she was dismissed with the right of defence.

## **3. Actuality of the topic and appropriateness of the set goals and tasks**

In recent years, there has been an increased interest in products of plant origin, which leads to an increased search for both new plant sources and a deeper study of already known medicinal

plants, with the aim of establishing the content of biologically active components or discovering unknown ones, current therapeutic applications and substances. In the context of modern approaches in phytochemistry, the selection of the subject of the dissertation *Trigonella foenum-graecum* L. seeds (Greek fenugreek) and the study of a possible application of the polysaccharide complex is very successful and is based on extensive preliminary studies. The wide range of primary and secondary metabolites reveals serious potential in various fields of application. The active components found in the seeds and their oil have therapeutic potential and a long history of traditional use. Despite the abundance of data on the beneficial effects of the seeds of the plant species in question, there is still a lack of specifics in the direction of characterizing the chemical composition of the essential oil and polysaccharide fraction contained in them.

In this regard, the idea of a phytochemical study of seeds from *T. foenum-graecum* cultivated in our country, as well as a study of the potential application of galactomannans isolated from it, is of essential scientific and practical importance, which undoubtedly demonstrates the relevance of the topic of the dissertation work.

#### **4. Knowing the problem**

Proof of the PhD student's knowledge of the problem is the literature review of the dissertation work, which is written on 49 pages with 190 literary sources, most of them are from the last five years. This illustrates a deep insight into the current state of the problem and the latest trends in the scientific field of the dissertation. The review is structured in 5 subsections. In the first and second subsections, the botanical characteristics of the species and the phytochemical composition of fenugreek seeds are examined in detail. In the third subsection, the pharmacological activity of the essential oil, galactomannans, saponins, alkaloids, and others biologically active substances isolated from fenugreek seeds was presented. In the following subsections of the literature review, the applications of fenugreek essential oil and galactomannans are described in detail. The literature review is well organized and the modern trends in phytochemical studies are logically summarized, which is proof of the good theoretical preparation and detailed knowledge of the problem by the PhD student.

#### **5. Research methodology**

The methods used for the isolation of a lipid fraction and subsequent analysis by gas chromatography with a flame ionization detector (GC-FID), spectrophotometric determination of the fatty acid composition, determination of tocopherols by high-performance liquid chromatography (HPLC), characterizing the composition of the essential oil by gas chromatography with mass detection, analyzing the monosaccharide composition of galactomannan by high-performance anion

exchange chromatography (HPAEC), Fourier transform infrared spectrometer (FTIR),  $^1\text{H}$  and  $^{13}\text{C}$  NMR are relevant and up-to-date. A method has been developed. for detection and validation of galactomannan by HPTLC. A method for obtaining of a film based on polysaccharide isolated from *T. foenum-graecum* was developed, and testing of a polysaccharide film for wound healing effect on buccal mucosa was carried out. The protocols for each methodology are detailed and comprehensively described. The PhD student has mastered a significant number of specific methods that are adequately applied, which is a guarantee of good and reliable results.

## **6. Characterization and evaluation of the dissertation work**

The presented dissertation is designed according to the requirements for dissertations of the Medical University of Plovdiv. The work contains 133 pages, 34 figures and 22 tables. The bibliography covers 190 titles. The dissertation includes the following sections: content, abbreviations used, introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusion, conclusions, contributions, list of scientific publications and participation in scientific events and projects, and literature. The structure of the individual parts is appropriate and logically arranged. The aim of the dissertation is clearly stated. Six tasks have been set for its practical implementation. The materials and methods used are described in detail. The results and their discussion constitute the main part of the paper. The results are precisely and accurately presented in 44 pages, illustrated with 15 tables and 22 figures.

The experimental results logically follow the implementation of the set tasks and were carried out in the following directions:

- Isolation and characterization of a lipid fraction from *T. foenum-graecum*. The lipid fraction was analyzed regarding the fatty acid and sterol composition, as well as the tocopherols.
  - Optimization the conditions for obtaining essential oil from fenugreek seeds and characterizing the chemical composition of the obtained essential oil. The method developed by Vanya Nalbantova allows the production of pure essential oil from plant material containing minimal amounts of oil, and the detection of biologically active components that cannot be obtained by classical methods. A comparative analysis of essential oil from fenugreek species cultivated in Bulgaria and India was carried out. As a result of the conducted research, significant differences were found in the composition of the oil, originating from Bulgaria and India.
- Optimization of conditions for isolation of polysaccharide fraction from fenugreek seeds. Optimizing the conditions of extraction of polysaccharide mixtures from the seeds of the studied taxon, and high yield and purity quantities were obtained.

- Investigation of the monosaccharide composition of the obtained polysaccharide fraction and establishment of the structure of the isolated substances. As a result of the conducted research, galactomannan was isolated and identified in a pure form, for which the ratio galactose:mannose 1:1 was confirmed. For the identification and quantification of the monosaccharide composition of hydrolyzed galactomannan, an HPTLC method was developed and validated, which is characterized by sensitivity, a high accuracy rate, and low limits of detection and quantification. In accordance with these characteristics, the proposed method is linear, accurate and reliable for the detection and quantification of galactose and mannose.
- Studying the possibilities of obtained pure polysaccharides for potential epithelizing action. The rheological characteristics of galactomannan isolated from *T. foenum-graecum* seeds were determined. As a result of the research, polysaccharide films were developed based on the isolated galactomannan and *Cotinus coggygria* Scop. extract, which were tested for wound-healing activity. Research shows that films containing a combination of galactomannan from *T. foenum-graecum* and an extract from *Cotinus coggygria* Scop. contribute to faster healing of lesions compared to controls and galactomannan plaques alone. The obtained results show good epithelizing properties of both fenugreek galactomannan and *Cotinus coggygria* leaves.

Seven conclusions are drawn. The conclusions are accurate, logical and cover the entire diversity of the obtained experimental data. Six contributions are listed. The literature reference includes 190 literary sources.

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

The contributions of the PhD thesis summarized by the PhD student are 6, which I could divide into 3 contributions of a scientific-theoretical nature and 3 of a scientific-applied nature. The PhD student for the first time characterized the chemical composition of essential oil from the seeds of *T. foenum-graecum* cultivated in Bulgaria. A comparative analysis of the terpene composition of essential oil from seeds cultivated in Bulgaria and India was also made. Galactomannan was also isolated and structurally identified from the polysaccharide fraction of the species.

I would pay special attention to contributions of a scientific and applied nature. An essential oil was isolated for the first time, using a newly developed method. For the first time, an HPTLC method for the identification and quantification of hydrolyzed galactomannan was developed and validated, and an epithelializing effect of a film coating of isolated fenugreek galactomannan in combination with sumac extract on rat buccal mucosa was tested.

#### **8. Evaluation of publications related to PhD thesis**

Related to the procedure, Vanya Nalbantova presents 4 publications, 3 of them with an impact factor, summarizing the most important results of her research. Vanya Nalbantova has presented messages on the topic of her dissertation at 2 national scientific forums and 3 international conferences for which she received two awards. She is a leading researcher on 1 scientific project and has carried out 1 international mobility at Institut Pascal, Université Clermont Auvergne, France.

#### **9. Personal participation of the PhD student**

I think that the contributions of the dissertation work are to a significant extent the work of the PhD student, of course under the expert guidance of her scientific supervisors - Assoc. Prof. Niko Yosif Benbasat, Ph.D. and Prof. Cedric Delattre, Ph.D. Vanya Nalbantova's personal contribution is confirmed by the fact that she is the first author in all presented publications and announcements from scientific forums.

#### **10. Summary of the dissertation**

The summary fully meets the requirements of the regulations of the Medical University-Plovdiv. It briefly and clearly reflects the achieved results. It is written on 49 pages, containing 12 tables and 17 figures. From the presented summary, one can gain a clear idea of the objectives, tasks, results obtained, discussion, conclusions and contributions of the dissertation work.

#### **11. Critical remarks and recommendations**

The dissertation is written in very good scientific language, well organized, thorough and interesting. The PhD student complied with all the recommendations made by me during the approval of the dissertation work, therefore I have no critical remarks and recommendations regarding the conducted research and the attached set of materials.

#### **12. Personal impressions**

I know PhD student Vanya Nalbantova from the preliminary defense of her dissertation work, which took place in September 2024. During her presentation, I was impressed by her confidence and competence in presenting the results of the scientific research conducted. Vanya Nalbantova's

participation in international mobility in France, as well as numerous qualification courses and training modules, left an extremely pleasant impression on a motivated young scientist who wants to upgrade and improve his knowledge and skills.

### **13. Recommendations for future use of dissertation contributions and results**

The obtained results are a solid basis for future research and for obtaining a pharmaceutical product with medical application. It would be interesting to deepen the pharmacological tests, as well as to apply the developed methods for obtaining essential oil and analyzing the monosaccharide composition on other plant taxa.

### **CONCLUSION**

The dissertation of Vanya Ivanova Nalbantova contains scientific and applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the Law and the relevant Regulations of the Medical University - Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements of the MU - Plovdiv. The dissertation shows that the PhD student Vanya Ivanova Nalbantova possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Pharmacognosy and Phytochemistry" by demonstrating qualities and skills for independent conduct of scientific 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 honorable scientific jury to award the educational and scientific degree "doctor" to Vanya Ivanova Nalbantova in a doctoral program in "Pharmacognosy and Phytochemistry".

25.10.2024

Reviewer:

/Asoss. Prof. Dimitrina Zheleva-Dimitrova, PhD/