

STATEMENT OF OPINION

by Prof. Dr. Stefka Vasileva Valcheva-Kuzmanova, PhD, DSc

Department of Pharmacology and Clinical Pharmacology and Therapeutics,
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of a dissertation for awarding the educational and scientific degree 'doctor'

professional direction **7.1. Medicine**

doctoral program **Pharmacology (including pharmacokinetics and chemotherapy)**

Author: Nikolay Borisov Yanchev

Form of doctoral studies: Full-time education

Department: Pharmacology and Clinical Pharmacology

Topic: Experimental pharmacological study of an extract from *Sideritis scardica*, Lamiaceae

Research supervisor: Assoc. Prof. Delyan Delev, MD, PhD, MHM, MU – Plovdiv

By Order of the Rector of the MU – Plovdiv P-983/19.04.2023, I was approved as a member of the Scientific Jury, and by a decision of the meeting of the Scientific Jury (Protocol №1/24.04.2023) I was chosen to prepare a statement of opinion on the procedure for awarding the educational and scientific degree "doctor" to Nikolay Borisov Yanchev.

1. General presentation of the procedure and the doctoral student

The presented set of documents in an electronic format is in accordance with Act on Development of Academic Staff in the Republic of Bulgaria, the Regulations for the implementation of the Academic Staff Development Act and the Regulations of MU-Plovdiv dated 28.01.2021 – Art. 70 (1) of Section I. Acquisition of educational and scientific degree "DOCTOR" at MU-Plovdiv

MPharm Nikolay Borisov Yanchev has presented 6 publications, of which 2 are referenced in Scopus. In this way, the minimum requirements of the Regulations for Academic Development at MU-Plovdiv (2021) have been fulfilled.

2. Brief biographical data for the doctoral student

Nikolay Borisov Yanchev was born on 22.08.1988. He is a master pharmacist since 2014 – he graduated from the Free University of Berlin, Faculty of Pharmacy. From 2014 to 2018, he worked as a master pharmacist in Germany, and from 2022 – as a master pharmacist at "C3I Europe" EOOD, Sofia. He was enrolled as a full-time doctoral student in the Ph.D. program Pharmacology (incl.

pharmacokinetics and chemotherapy) at the Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, Medical University of Plovdiv by Order of the Rector of the Medical University of Plovdiv P-331/28.02.2019.

He speaks German, English and Russian. Has completed courses and trainings: SPSS statistical software package, Protection and welfare of experimental animals used for scientific or educational purposes.

3. Relevance of the topic and appropriateness of the set goals and tasks

Although medicinal plants are widely used in folk medicine, only a small number of them have been experimentally and clinically tested. The study of the composition of medicinal plants, as well as the experiments with them, aim to lay the scientific foundations of their traditional use in folk medicine.

In this sense, the study of *Sideritis scardica* (Mursal tea) has important contributions of scientific and scientific-applied value. The dissertation is the first systematic pharmacological study of this species endemic to Bulgaria.

The aim was to carry out a systematic study of the toxicity and various pharmacological effects of an extract from mursal tea (*Sideritis scardica*) – anti-inflammatory, anxiolytic and neuroprotective.

To achieve this goal, 7 very voluminous tasks have been set, which include phytochemical analysis of the extract, determination of its antioxidant activity, study of acute and chronic toxicity, as well as establishing the effects listed above by using multiple behavioral, histopathological and biochemical indices.

4. Knowledge of the problem

MPharm. Nikolay Yanchev has made a very thorough review of the literature in several aspects: 1) Chemical composition of *Sideritis scardica*, highlighting the substances in it that are in the greatest concentration and would be important for its pharmacological activity; 2) Current research on the effects of *Sideritis scardica* on the CNS; 3) Plant toxicity, potential for interactions and biotransformation; 4) Anti-inflammatory activity of plants of the genus *Sideritis*; 5) Stress with its harmful consequences for the normal functioning of the body; 6) Cytokines in stress models and an inflammation model by injection of lipopolysaccharide; 7) Models of cognitive deficits and neurodegenerative changes in the CNS.

The detailed literature review enables Nikolay Yanchev, under the competent guidance of his scientific supervisor, Assoc. prof. Delyan Delev, to direct his research work to the plant *Sideritis scardica*, for which there are no systematic pharmacological studies. The main interest is in the neuroprotective and anti-stress effects of the herb with special emphasis on pro-inflammatory cytokines as markers of stress and inflammation.

5. Research methodology

The research related to the dissertation includes an extremely diverse methodology. There are several groups of methods: I. Extraction and phytochemical analysis; II. In vitro determination of the antioxidant activity of the extract; III. Studies in experimental animals. The last group of methods includes: 1. Determination of acute and chronic toxicity of the extract; 2. Immunological analysis of cytokines in models of acute cold stress and chronic stress, as well as in a model of chronic inflammation caused by lipopolysaccharide; 3. Studies in 2 models of stress – acute cold and chronic, as well as in 3 models of induced memory impairment – with diazepam, scopolamine and sodium nitrite; 4. Behavioral tests performed in the stress and impaired memory models: forced swim test, elevated plus maze test, social interaction test, locomotor activity in an activity cage apparatus, T-maze test, recognition test, Y-maze, active learning method (Two-way active avoidance test; Shuttle-box test), passive learning methods (step-through and step-down); 5) Studies in a rat hindpaw edema model induced by carrageenan administration; 6) Statistical methods – data processing using IBM SPSS 19.0 software package.

Very reliable and informative methods and models for establishing the pharmacological effects of *Sideritis scardica* extract have been chosen, which make it possible to draw adequate conclusions in response to the research tasks. The mastery of this extremely large number of pharmacological methods is a prerequisite for a very successful future research work of Nikolay Yanchev.

6. Characterization and evaluation of the dissertation

The dissertation is well structured and includes the following sections: Introduction – 2 pages, Literature review – 31 pages, Aim and tasks – 1 page, Scientific idea and working hypothesis – 1 page, Material and methods – 11 pages, Results – 28 pages, Discussion – 19 pages, Summary – 1 page, Conclusions – 1 page, Contributions – 1 page, Appendix – 15 pages, Bibliography – 28 pages, including 303 sources in English, List of publications and participation in scientific forums – 2 pages.

With the comprehensive and very targeted literature review the author directs the reader to the aspects of the research work carried out. The knowledge of the literature enables Nikolay Yanchev

to direct his work to hitherto unexplored effects, which contributes to the originality of the obtained results.

The goal and tasks are clearly formulated and require extremely diverse and very voluminous research work.

The description of the materials and methods shows that Nikolay Yanchev knows them well and proves to his personal engagement in the experimental work.

The research results are presented in detail and illustrated with 39 tables in the main text and 30 figures in the Appendix. Values are presented as mean \pm SEM. Statistical significance is indicated, which was determined with IBM SPSS 19.0 software by ANOVA test, Levine test for homogeneity of distribution between groups, and the results for significance are presented by post-hoc test LSD in the presence of homogeneity and Games-Howell in the absence of homogeneity. Results are considered statistically significant at a significance level of $p < 0.05$. Correctly performed statistical processing of the experimental data enables correct and reliable conclusions from the studies.

Important results of an original character, with scientific and scientific-practical value, were obtained. The effects recorded in the own studies are skillfully discussed, indicating the possible mechanisms behind these effects in accordance with the data known so far in the scientific literature.

The results are very clearly summarized in the Summary of the dissertation.

Based on the precisely conducted and described experiments, 7 conclusions were drawn that correctly reflect the results of the research, highlighting the established antioxidant and anti-inflammatory activities of *Sideritis scardica* extract, as well as its ability to reduce anxiety and improve memory.

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

Important contributions of scientific-theoretical and scientific-practical importance have been obtained. For the first time, in models of acute and chronic stress, an anxiolytic effect of *Sideritis scardica* extract has been demonstrated, and in various models of memory impairment, a neuroprotective effect of the extract has been established. For the first time in an experiment, a correlation between the development of anxiety and increased levels of pro-inflammatory cytokines has been found. Studies have shown a dose-effect relationship of *Sideritis scardica* extract.

The experimental studies carried out with the extract of *Sideritis scardica* are a prerequisite for conducting clinical studies with it.

8. Assessment publications related to the dissertation

MPharm. Nikolay Yanchev has applied 6 publications in relation to the dissertation work, 2 of which are referenced in Scopus.

The research results have been popularized through 6 participations in scientific forums, of which 3 are international and 3 are national.

9. Personal participation of the doctoral student

MPharm. Nikolay Yanchev is the first author of all publications and abstracts from participations in scientific forums. This shows his great personal contribution for the conducted dissertation research as well as for the results obtained.

10. Abstract

The abstract is written in 54 pages, made according to the requirements and reflects the main results achieved in the dissertation. The results and discussion are well illustrated with 35 figures and 6 tables.

11. Critical remarks and recommendations

I have no critical notes. The materials submitted for review are very well organized and timely presented.

12. Personal impressions

I have no prior personal impressions of MPharm. Nikolay Yanchev, but the implementation of such a large set of experiments, as well as the interpretation of the obtained results, show systematicity, order and logic.

13. Recommendations for future use of dissertation contributions and results

I recommend to MPharm. Nikolay Yanchev in his future development as a researcher and scientist, to apply the numerous pharmacological methods he learned, which is a prerequisite for his professional success.

CONCLUSION

The dissertation contains scientific-theoretical and scientific-practical results and contributions that meet all the requirements of Act on Development of Academic Staff in the Republic of Bulgaria, the Regulations for the implementation of the Academic Staff Development Act and the Regulations for Academic Development at MU – Plovdiv (2021). The presented materials and dissertation results fully correspond to the specific requirements of the MU – Plovdiv.

The dissertation shows that the MPharm. Nikolay Yanchev has in-depth theoretical knowledge and professional skills in Pharmacology which are a prerequisite for future independent planning and conducting of scientific research, as well as for interpreting the results. The publications presented in relation to the dissertation show the great personal involvement of the doctoral student, competently supervised by Assoc. prof. Dr. Delyan Delev.

Due to the above, I confidently give my **positive assessment** of the conducted research, presented by the dissertation work and abstract, and I propose to the honorable scientific jury to award the educational and scientific degree "doctor" to **Nikolay Borisov Yanchev** in the doctoral program **Pharmacology (including pharmacokinetics and chemotherapy)**.

Заличено на основание

Чл. 5 §1, б. "В" Регламент (ЕС) 2016/679

18.05. 2023

Member of the Scientific Jury:.....*[Signature]*.....

Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc