

REVIEW

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of the Doctoral Thesis presented for awarding the degree “**Doctor of Chemistry**” in the Field of higher education 4.0. "Natural Sciences", Professional Field 4.2. "Chemical Sciences", scientific specialty „*Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances*”

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Ms. Ivanova's dissertation is dedicated to the phytochemical study of three species of the genus *Inula*, growing in Bulgaria, incl. a subendemic species. Some members of this genus are well-known as medicinal plants with a variety of useful properties in the traditional medicine of Asian nations. Plants of the genus *Inula* are rich in biologically active secondary metabolites of different structural classes, and data on the species selected for the present study of Bulgarian origin are scarce. In view of the growing interest in healing molecules from natural sources, the topic of the work is important and relevant. It is also an up-to-date and valuable continuation of the long-term research of the Laboratory of Natural Product Chemistry – IOCCP, on the Asteraceae family.

The dissertation is structured according to the classical scheme. The literature review includes 35 pages, it covers the available data on the chemical composition of the three studied species: *I. britannica*, *I. oculus-christi* and *I. aschersoniana*. Special attention is paid to the sesquiterpene lactones which are characteristic of the genus, and to flavonoids and phenolic acids, to the structural diversity of these compounds, to the methods of their isolation and structural elucidation. The biological properties of extracts from the three plants, as well as the methods for assessing the antioxidant activity are summarized. The candidate was able to summarize and organize in appropriate tables a large amount of information. The literature review shows her

excellent literary awareness of the studied problem and allows her to formulate the goals and objectives of her dissertation in order to fill the existing gaps in the knowledge of the studied taxa.

The chapter dedicated to the research results covers 40 pages. Here, a detailed description is provided of the isolation of individual compounds by a variety of modern chromatographic techniques, as well as their structural characterization using up-to-date spectral methods - UV spectroscopy with shift reagents, high-resolution mass spectrometry (ESI-TOF), ^1H - and ^{13}C - NMR spectroscopy (one- and two-dimensional techniques - COSY, HCQC, HMBC, NOESY). More than 30 compounds have been isolated and identified - sesquiterpene lactones, sesquiterpenoids, triterpenoids and sterols, phenolic acids and flavonoids. The structure and, in most cases, the stereochemistry of the isolated compounds has been unambiguously and convincingly proven, using all available spectral and chemical data. The PhD candidate has successfully coped with the serious challenges offered by the molecules of the 9 newly discovered natural compounds, of course with the necessary assistance and guidance from the supervisor Prof. A. Trendafilova. I particularly appreciate the structural characterization of the two new compounds containing cyclopropenone ring. This is a significant scientific contribution, as so far only 6 compounds with this structural element are known in nature. The comparison of the obtained data on the chemical composition of the three species with each other and with the literature data lead to some chemotaxonomic conclusions.

The next stage of the research is the comparative qualitative and quantitative analysis of the extracts from 11 different Bulgarian localities of the plant species most commonly used in medicine - *I. britannica*, to characterize intraspecific variability and to indicate promising populations rich in biologically active components - phenolic compounds and/or sesquiterpene lactones. Sesquiterpene lactones were analyzed by gas chromatography, and HPLC was used to quantify chlorogenic and isomeric dicaffeoyl esters of quinic acid, and appropriate quantitative methods were developed. The potential of these extracts to scavenge DPPH $^{\bullet}$ and ABTS $^{\bullet+}$ free radicals was also evaluated. These results are definitely a merit of the dissertation, with a perspective for practical application, as they allow to identify populations rich in valuable bioactive components.

The section "Experimental part", presented on 15 pages, describes the methodological approaches used in the work, which are up-to-date and appropriate.

In general, the dissertation is written concisely, clearly and logically. I have some small remarks as follows:

- On fig. 15. the structure of anthocyanidin is not correct, it lacks the double bond and the positive charge on the oxygen atom in cycle C.
- In the structural elucidation of compounds 173 and 174, it should be noted that they are newly discovered natural compounds.
- Compared to the detailed explanation of the spectra of known compounds in the dissertation, comments on the structural elucidation of Ashersonians A and B are quite scarce. As these are new compounds of a rare structural type, they deserve more in-depth discussion.
- On page 67 there is a statement that "(173 and 174) are 3-angeloyl and 3-senecyoyl esters of the same aglycone." In fact, it is the alcohol component of the esters, not the aglycone.
- In Table 12, it would be good to present the yield of the extract as percentage.

These notes are mainly of a technical nature and do not reduce the value of the presented dissertation. I have the following questions to the PhD Candidate:

- From which sources have been the other known natural compounds with cyclopropenone ring isolated?
 - Why are only caffeoylquinic acids quantified by HPLC but not flavonoids?
- According to the data in table 10 the amount of total flavonoids in some cases is comparable to that of total phenolics.

It should be noted that the PhD Candidate has performed a large amount of experimental work, presented the results correctly and commented on them competently. She has mastered various modern methods - techniques for isolation and purification of secondary metabolites from plant material, spectral methods and approaches for structural characterization of small natural organic molecules,

methods for studying radical scavenging activity. This convincingly demonstrates that the educational goal of the dissertation has been successfully fulfilled.

The main contributions of the dissertation are scientific and to some extent applied scientific. They can be characterized as a novelty for science and as an extension of existing knowledge, and are a valuable contribution to the knowledge of medicinal plants and their biologically active secondary metabolites. The abstract reflects correctly and comprehensively the content of the dissertation and can be accepted without any remarks.

Part of the dissertation results has been reported in 6 scientific papers published in journals indexed in the Web of Science and/or Scopus, one of which - *Phytochemistry Letters* - in the first quartile (Q1). Some of the results were presented at seven international scientific forums. A total of 16 citations have been noticed so far on 4 of the published works.

I have known Ms. Victoria Ivanova personally since she was admitted to the lab. Chemistry of Natural Products of IOHCCP and I have very good impressions of her as a diligent and experienced experimenter, well acquainted with the literature on the issues on which she works. I am convinced that the contributions to the work are mainly her own, of course with the necessary support and guidance from her supervisor. Ms. Ivanova was also the coordinator of a project funded by the National Research Program "Young Scientists and PhD Students", which reinforces the impression of her leading role in research.

CONCLUSION

Taking into account all the above, it can be concluded that Ms. Ivanova's dissertation is a serious and in-depth up-to-date research, with significant contributions in the field of studying Bulgarian medicinal plants and the possibilities for their use. The dissertation fully meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, and the regulations for its application, as well as the specific requirements of IOCCP - BAS for the required educational and scientific degree. In the course of the research the Candidate has acquired diverse knowledge and skills in the field of chemistry of

natural products. This gives me reason to vote positively with deep conviction for the award of Ms. Victoria Ivanova to the educational and scientific degree "Doctor" in professional field 4.2 Chemical Sciences, and to recommend to the esteemed Scientific Jury to do the same.

23rd June, 2022

Reviewer:

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