REVIEWS

by Paraskev Todorov Nedyalkov, PhD - Associate Professor at the Faculty of Pharmacy at the Medical University of Sofia

of the materials submitted for the competition
to occupy the academic position of "Associate Professor"
in Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS.
in area of higher education 4. Natural sciences, mathematics and informatics
professional field 4.2. Chemical Sciences
(01.05.10 Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances)

In the competition for „Associate Professor", announced in the State Gazette, issue. 43 of May 31, 2019 and on the website of IOCCP, BAS, as candidate participates Assistant Professor Boryana Stoykova Trusheva, PhD from the Laboratory for Chemistry of Natural Substances at the IOCCP, BAS.

1. General evaluation of the applicant's materials

The **only person** applied for the competition was:

Assist. prof. Boryana Stoykova Trusheva, PhD from the Laboratory for Chemistry of Natural Substances at the IOCCP, BAS.

The set of paper-based materials submitted by assist. prof. Boryana Trusheva is in accordance with the Rules for the Development of the Academic Staff of IOCCP, and meets the criteria of IOCCP-BAS for occupying the academic position "Associate Professor".
The candidate Boryana Trusheva has enclosed a total of 35 scientific publications, of which 3 are parts of books, one is a registered utility model and the other 31 are scientific articles. Thirty scientific works that are outside the PhD thesis will be subjected to review. All these scientific works are on the subject of the competition. The distribution of scientific papers by the relevant Q factors is as follows: there are 8 publications published in Q1 journals; 9 in Q2 journals; 3 in Q3 journals and 1 in Q4 journals. Of the above 30 peer-reviewed scientific papers 7 are included in habilitation work (indicator C), equivalent to 135 points, 18 works (equivalent to 372 points) are included in indicator D and 3 publications have no impact factor or impact rank.

2. Brief biographic notes

Assist. prof. Boryana Trusheva has obtained her master degree at the Faculty of Chemistry at Sofia University "St. Kl. Ohridski" under the Master's Program in Organic and Analytical Chemistry. In the time period 2002-2006 she has done her PhD thesis in 01.05.10. "Organic Chemistry, Chemistry of Natural and Physiologically Active Substances" with the title "Chemical composition and biological activity of propolis from different geographical regions" at the IOCCP, BAS. Her professional career began as a chemist in 2006 at the IOCCP, BAS, and in 2007 she occupied the academic position "Assistant Professor".

3. General characteristics of the applicant's activities

The applicant assist. prof. Trusheva has presented 34 publications in scientific journals and books as well as one useful model equivalent to a publication.

Five of the publications were used by the candidate for her PhD thesis and these are also not included in the indicator C and D lists and will not be reviewed.

The candidate included in indicator C 7 of her publications with an impact factor (the total impact factor is 10.718). All works in this indicator fully comply with the national and institutional regulatory requirements and according to the Scopus and Web of Science rating they can be classified as follows: in Q1 falls 1 publication; in Q2 there are 4 and in Q3 are 2. Thus, the included in indicator C articles five 135 points with a required minimum of 100 points. Assist. prof. Trusheva is the first author in five of the publications while in the other 2 she is the second author. All publications on this indicator have three or more authors and the candidate is author for correspondence in 3 of them.

A total of 18 works are included in indicator D, which is in full agreement with national and institutional regulatory requirements. Fourteen publications have been published in journals with impact factor (total IF 27.228) that are distributed by quartile as follows: 7 publications in Q1 magazines; 5 in Q2; 1 in Q3 and 1 in Q4. In addition to these publications, 3 book chapters and 1 recognized utility model request are included in this indicator. The total number of points formed under indicator D is 372, while the required minimum is 220 points. Only one of the included publications in this indicator has two authors, while the other have three or more authors. The candidate assist. prof. Boryana Trusheva is the second author in 7 of the publications, while in the rest articles she is in the third or more backward position.

The teaching and pedagogical activity of assist. prof. Boryana Trusheva is involved in the training of graduates and interns. She was the supervisor of one graduate student and one intern, and she was also a consultant to two graduate students.

The applicant's contributions can be classified as scientific, applied and methodological.

In indicator B 7 publications has included, as mentioned above. All scientific papers in this indicator are related to research on propolis. Five of these describe studies whose contributions are predominantly scientific and the other 2 are applied science in nature. Scientific publications are related to the studies of the chemical composition and biological activity of propolis from collected in Iran (Isfahan Province), Indonesia (East Java), Brazil, Taiwan and some Pacific Islands (Fiji Islands and Pitcairn Islands). The scientific contributions from these studies can be classified into the following groups:

- Isolation and structural elucidation of new natural products and identification of new for propolis compounds;
- Analysis of the essential oil composition of propolis;
- Evaluation of biological activity of propolis and its constituents.

Six new natural compounds were isolated and structurally elucidated: two prenylated stilbenes with an unusual sesquiterpene side chain, solomonin B and solomonin C, from propolis collected on Fiji island and 4 cycloartane triterpenes 3-oxo-cycloart-24*E*-en-21,26-diol-21,26-diacetate, 3-oxo-cycloart-24*E*-en-21,26-diol, 3-oxo-cycloart-24*E* -en-21,26-diol-21-acetate and 3-oxo-cycloart-24*E*-en-21,26-diol-26-acetate from propolis localized at o. Pitcairn. The structures have been established by means of modern spectral techniques such as 1D and 2D NMR experiments, UV spectroscopy and high-resolution mass spectrometry. The spectral data have been presented and commented correctly and have

been given in the required volume. In addition, 14 known compounds were found in propolis for the first time: 1 fatty acid (9-oxo-10 (E) -12 (Z) -octadecadienoic acid), 1 prenylated coumarin, 1 prenylated flavonoid, 4 alk(en)ilresorcinols, 4 terpene esters and 3 triterpenes of cycloartane type. Most of the compounds have been isolated and their structure has been established by the above mentioned spectral techniques, while others have been identified using GC-MS. To this group of scientific contributions can be added 2 works that were included in indicator D. These are related to the chemical composition and biological activity of propolis. For the first time, a propolis from Malta was investigated, from which two novel propolis natural products were isolated and identified: 2-acetoxy-6-p-methoxybenzoyl jaeschkeanadiol and 2-acetoxy-6-p-hydroxybenzoyl jaeschkeanadiol. These components are specific of the species *Ferula communis* (family Apiaceae), which is the most likely secondary plant source of Maltese propolis. Furthermore, the diterpene profile of propolis from Malta and Greece was compared with that in *Cupressus sempervirens* and *Pinus halepensis* resins collected from Malta. According to the results, the main and/or the only plant source of the Mediterranean type propolis is the cypress *C. sempervirens*, and this is the first report of cypress as a plant source of propolis.

The volatile fractions obtained from Brazilian red propolis and Taiwanese green propolis were analyzed by GC/MS and it was found that the main volatile components of Brazilian red propolis, that have been poorly studied, were the phenylpropanoids: elemicin, methyl eugenol, transmethyl isoeugenol, isoelemicin and trans-anethole. While the major constituents of Taiwanese green propolis essential oil, which has not been studied so far, were: β-eudesmol, 6-methyl-3,5-heptadiene-2-one, γ-eudesmol, geranial and 6-methyl-5-heptene-2-one.

Studies on radical, antibacterial, antifungal and antitumor activity of some of the isolated compounds and propolis extracts have been performed. Radical-scavenging activity against diphenylpicrylhydrazyl radicals (DPPH) of prenylflavanones propolins C, D, F, and G. isolated from Iranian propolis has been established. Antitumor activity on human breast cancer cells (MDA-MB-231) of propolis extract collected from Pitcairn Island has been established. This extract has shown antibacterial activity against *Staphylococcus aureus* along with the terpenic esters isolated from Iranian propolis esters chimgin, chimganin, ferutinin and teferin. It has also been found that the extract from a propolis collected on Pitcairn Island and some of its new cycloartan triterpenes are active on *Escherichia coli* and *Candida albicans*. The two contributions included in indicator D can also be attributed to the scientific contributions to propolis biological activity research. The activity of propolis components on the bee pathogen *Paenibacillus larvae* was examined for the first time. The flavonoids pinocembrine and 3-O-acetyl-pinobaxin, as well as the caffeic acid ester mixture (pentenyl, isopentenyl and phenethyl caffeate) have been found to possess the highest antibacterial activity.

Applicant's scientific contributions include 2 papers that have not been included in indicator B and are related to studies of woody mushrooms from the Basidiomycetes class. From the tree fungus *Fomitopsis rosea*, 2 new natural triterpenes [3α- (3′-butylcarboxyacetoxy) oxepanoquercic acid C and 3α-hydroxy-24-methylene-23-oxol-8-ene-26-carboxylic acid] were isolated, together with three known lanostolic triterpenes and epidioxy sterol. All isolated compounds exhibit activity against *Staphylococcus aureus*. GC/MS analysis of the potentially cytotoxic chloroform extract from macromycellar fungus *Hygrophorus agathosmus* has led to identification of several hydrocarbons, including paraffins, pier, phytane and squalene. The sterol composition of this mushroom was analyzed for the first time by GC/MS and the main sterol being ergosterol

(22E-ergosta-5,7,22-triene-3β-ol) (82%), along with two other ergostane derivatives: (22E)-ergosta-5,7,9(11),22-tetraene-3β-ol (11.5%) and ergosta-7-ene-3β-ol (6.5%). 5α, 8α-Epidioxi-24(ξ)-methylcholesta-6,22-diene-3β-ol was isolated for the first time from *H. agathosmus* but its presence might be regarded as an artifact obtained by oxidation of the corresponding $\Delta^{5,7}$ sterol.

The contributions included to indicator B with applied science nature relate to studies on propolis extraction techniques and nanofiltration of propolis extract. The classical extraction, microwave and ultrasonic assisted extraction of propolis were studied under varying conditions (extraction time, crude propolis / solvent ratio) as well as a monitoring of the amounts of extracted phenols and flavonoids. This study has found that the most effective method in terms of yield, extraction time and selectivity is ultrasonic extraction. For the first time, a *dead-end* nanofiltration has been successfully applied as a method of concentrating biologically active substances derived from poplar propolis. Retention of more than 95% of all analytes and concentration of propolis extract more than three times was achieved using Duramem ™ 200 membrane.

Four publications and one utility model included in indicator D could be also related to the contributions with applied science nature. These are related to the production and characterization of water-soluble propolis, loaded with propolis polymeric micelles and modified with MCM-41 and SBA silver -15 mesoporous materials that are suitable carriers of poplar propolis. The new propolis-loaded forms and carriers have been shown to exhibit very good *in vitro* cytotoxic and antibacterial activity. The quantitative characteristics of Bulgarian propolis were examined for the first time. Minimum values for the content of biologically active substances in crude poplar propolis are proposed. These values may serve as a basis for propolis standardization.

One publication included to indicator D can be related to methodological contributions. An overview of the available methods for studying propolis in different aspects is presented for the first time (including based on our experience): propolis in the bee colony, chemical composition and plant sources of propolis, biological activity of propolis with respect to bees and humans, and approaches for standardization and quality control for the purposes of industrial application.

Seven of the applied papers (6 are included in indicator D) are reviews on propolis and one is on the phenylethyl ester of caffeic acid. These articles and book sections provide a thorough overview of the plant sources of propolis from different geographical and climatic regions, as well as the chemical composition and biological activity of propolis. Particular attention is paid to the newly discovered natural and propolis components. The literature data on volatile compounds and essential oils in propolis from different geographical areas have been summarized. A thorough analysis of the literature data rejects the widely accepted claim that essential oil is a major component of propolis. Regardless of geographical origin, propolis typically contains no more than 1% of essential oil, in rare cases up to 2-3%. Data on recent applications of propolis except those related to improving and protecting human health have also been summarized. A review of current data on the application of metabolic approaches and chemometrics has also been made to investigate propolis and its standardization problems. A recently published review on phenylethyl ester of caffeic acid examines extensively the appeared after 2014 year literature data on this compound. Particular emphasis is placed on the natural sources of this compound, the analytical aspects and methods for its synthesis.

The works of assist. prof. Boryana Trusheva find a wide international response, as evidenced by the numerous citations. She listed a total of 1,229 citations in refereed (778) and in non-refereed (451) sources. At the time of writing this review, 693 citations were appeared in Scopus and 653 citations in Web of Science. According to the regulations of IOCCP-BAS, the applicant exceeds by far the minimum requirements for indicator E regardless of which source it be considered. Again, under these Rules of IOCCP-BAS, the applicant also meets the requirements of indicator G, where for the academic position "associate professor" the minimum requirement of h-index is 5, while Dr. Boryana Trusheva has 15.

The most of the research that Dr. Trusheva's was involved in were subjects of 11 research projects funded by national and international sources. Along with them, the applicant participated as an expert in commercial contracts.

The guidelines for future research formulated by Dr. Boryana Trusheva, which she will work on over the next few years, are as follows:

1. Continue the research on propolis, and in particular propolis from the stingless bees.

2. Perform studies related to the use of natural deep eutectic solvents as green solvents for extraction of biologically active compounds from natural sources, including propolis.

3. Continue the studies on the synthesis of phenethyl ester of caffeic acid.

4. Assessment of the applicant's personal contribution

In nearly half of the publications, assist. prof. Boryana Trusheva is the first or second author, and in three of them she is the author of correspondence. It is a strong evidence that she plays a significant role in the design and implementation of the experiments and in the writing of the manuscripts of these publications.

5. Critical comments and recommendations

I have no substantive remarks on the enclosed works. The habilitation report must be a summarized, systematic, synthesized and brief presentation of the applicant's most important contributions. However, the enclosed habilitation report is rather a brief abstract of the candidate's works with which she participates in the competition. I also have some comments on the attached publication lists. Only one list is needed, where the works should be classified in the following categories: 1). works used to acquire the PhD degree; 2). jobs equated to habilitation work (indicator B); 3). publications in indicator D; 4). works that the applicant considers important but cannot be classified in the above categories.

CONCLUSION

The documents and materials presented by assist. prof. Boryana Stoykova Trusheva **meet all the requirements** of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Rules for the implementation of the LDASRB, the Rules for the Implementation of the LDASRB of BAS and the Rules of the IOCCF-BAS.

The candidate submitted **a sufficient number of scientific papers** published after the materials used in her PhD thesis. The candidate's works contain original scientific and applied contributions that have received international recognition and a representative part of them have been published in journals and scientific books published by international academic publishers.

Her theoretical developments have practical applicability. The scientific qualification of assist. prof. Boryana Stoykova Trusheva is **undoubted**.

The results achieved by assist. prof. Boryana Stoykova Trusheva in her research activity **fully comply** with the specific requirements of the IOCCF-BAS regulations for the implementation of LDASRB.

After getting acquainted with the materials and scientific works presented in the competition, an analysis of their importance and the contributions with scientific and applied scientific nature contained therein, I find it reasonable to give my **positive assessment** and to recommend to the Scientific Jury to prepare a report proposal to the Scientific Council of IOCCF-BAS for the promotion of assist. prof. Boryana Stoykova Trusheva in the academic position of Associate Professor at IOCCF-BAS in the professional field 4.2. Chemical Sciences (scientific specialty 01.05.10 "Organic Chemistry, Chemistry of Natural and Physiologically Active Substances").

09.09.2019 г.

Reviewer: .....

Assoc. Prof. Paraskev Nedialkov, PhD