—

# REFEREE REPORT

By Prof. Pavlinka Aleksandrova Dolashka, D.Sc. from Institute of Organic Chemistry with Center of Phytochemistry – BAS,

the submitted materials for participation in the competition for academic position 'Professor' at the Institute of Organic Chemistry with the Center of Phytochemistry - BAS (IOCCP), by professional field 4.2. Chemical Sciences, specialty 01.05.10 "Organic Chemistry, Chemistry of Natural and Physiologically Active Substances". for the needs of the laboratory "Chemistry of natural substances" from 09.2018 at the Institute of Organic Chemistry with the Center of Phytochemistry – BAS.

In the competition for the academic position 'Professor' announced in the State Gazette. pcs. 43, page 122, May 31, 2019 and on the web site of IOCCP, BAS, participated only one candidate, Assoc. Prof. Dr. Milena Petkova Popova from IOCCP -BAS.

## 1. General presentation of the received materials

For the participation in the announced competition have submitted documents from a single candidate Assoc. Prof. Milena Popova Popova.

The materials, presented for reviewing, completely correspond to the requirements of the Law for Scientific Development in Bulgaria, the Rules of its Application as well as to the Internal Rules and Regulations of the Institute Organic Chemistry with Center for Phytochemistry (IOCCP) for acquiring the academic position "Professor".

Pack of documents for reviewing: Completed table (Annex 1 of the Rules) certifying the meeting of the requirements for occupying the academic position of "Professor"; 2. Scientific autobiography; 3. Diploma of educational and scientific degree "Doctor"; 4. Diploma for the academic position "Associate Professor"; 5. An abstract of the dissertation for the educational and scientific degree "Doctor"; 6. Extended habilitation reference for the scientific contributions of Bulgarian and English with a common list of publications; 7. List and copies of publications by Indicator B (equivalent equivalent number articles); 8. List and copies of publications and certificate of registration useful model by indicator D; 9. List of participations in

scientific events with attached evidence; 10. List of quotations not submitted in another competition; 11. List of participations in research projects with attached evidence material; 12. Additional information about the scientific activity - list and evidence material.

## 2. Biographical data

Assoc. Prof. Milena Popova is PhD graduate of the Sofia University “St. Kliment Ohridski”, Faculty of Chemistry in 1998 with specialty “Chemistry and physics”. She continued her education in the IOCCP-BAS, where she acquired a PhD degree (2004) in the scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active substances”. Dr. Popova is a head of a scientific team on applied research in the field of biochemistry, food and pharmacy - Laboratory of Chemistry of Natural Compounds in the Institute of Organic Chemistry with Center of Phytochemistry - BAS,

## 3. General characteristics of the applicant's activities

*Evaluation of the applicant's scientific and applied scientific activity*

Dr. Popova is an author of 77 scientific papers, 32 of which are included in the application for the academic position “Professor”, and 2 publications, published in books abroad. Her papers have been quoted 1172 times. She is also a co-author of 1 useful model in the field of pharmaceutical biotechnology. She participated in 8 scientific projects with national funding and 9 international scientific project and guide 2 national scientific project.

*Assessment of educational and pedagogical activity*

Dr. Popova was a head of two defended graduate students from Bulgaria and participated in the training of four graduate students from abroad (Malta and Thailand - joint publications).

*The scientific contributions*

The scientific contributions of Dr. Popova are in the field of chemistry of natural and physiologically active compounds, revealing their composition, structure of active compound, structure-biological activity relationship, development of methods for standardization and quality control of these bio-compounds. The main research objects of laboratory are the medicinal plants, mushrooms, propolis, and the bee product of plant used in folk medicine for prevention and treatment of various diseases.

The scientific contributions of Dr. Popova's research work have both fundamental and applied character and are directed mainly to studying the chemical composition of medicinal and economically important plants and their biological activity.

The scientific and applied contributions from the presented 35 publications can be summarized in two thematic areas:

Thematical area I. Studies on propolis
Thematical area II. Studies on medicinal plants

The main topic of the scientific research of Dr. Popova is the Thematical area I. Studies on propolis, which includes two subareas:

1. Investigation of the chemical composition and biological activity of propolis from different geographic regions and bee species, and its plant source determination;
2. An overview of propolis data.

The different plant secretions contain the main compounds, and biological properties of propolis, depending on the plant biodiversity in different geographic and climatic regions.Due to its complex and diverse chemical composition and its difficult standardization, propolis is a subject of intensive research.

*The first subarea:* 1. Investigation of the chemical composition and biological activity of propolis from different geographic regions and bee species, and its plant source determination.

Dr. Popova has studied the chemical composition of over 20 propolis types classified into three major groups - originating from temperate, subtropical and tropical regions. She presents very important data about propolis from temperate regions in Europe and North America, from subtropical climatic regions from Europe and North Africa and from tropical regions in Asia, South Pacific islands, Africa, South America and East Asia.

Several propolis and individual components produced by the honey bee *Apis mellifera* L. (tribe Apini), which inhabit almost all ecosystems of the world and by the stingless bees (tribe Meliponini) - the native bee species in tropical and southern subtropical regions, were analysed using the most common approaches as GC/MS (after derivatization).

*Propolis from temperate regions (within Europe, North America, Argentina, Southern Africa, Asia and New Zealand)*

The presented results from Dr. Popova revealed a similar chemical composition with main biologically active components (flavonoids, phenolic acids and their esters) in propolis from temperate regions. The most intensively studied propolis is mainly from *Populus nigra* L. Propolis, plant source from Europe. She has offered new methods and criteria for its quality control and standardization, which have been accepted and recommended by the International Honey Commission for standardization purposes.

Very important information, obtained after analyzing 22 samples by GC/MS of Bulgarian propolis from different regions, presented it as a high quality product. After detailed chemical analyses of propolis from Poland, she also presents more than 80 compounds with antitumor and antimicrobial activity.

Investigation of the chemical composition of propolis from healthy and from bee colonies infected with Varroa destructor and pathogen *Paenibacillus larvae* helps to solve the main stress factors contributing to the high levels of bee colony losses in the world. Dr. Popova has also found higher content of caffeic acid and its pentenyl esters in the samples of healthy colonies in comparison to the bee with *V. destructor* infection. The reason for all of these observations is unclear, but it is a very important problem to be solved.

The information about the composition and properties of propolis from subtropical climatic regions was supported by the analyses of propolis from North America. The inhibitory activity and the chemical composition of 10 propolis samples from different regions of America are presented by the author. Based on 60 components identified by GC/MS, she classified the samples into three groups: rich in aromatic acids, new for propolis ethyl ether of coumaric alcohol, triterpenic acid and flavonoids. A relatively high content of aromatic acids was identified in the poplar propolis originating from Populus tremuloides (American aspen). As the most probable source of the samples with high flavonoid content and inhibitory activity against Cromobacterium violaceum strain the poplar *P. fremontii*, was identified.

*Propolis from subtropical climatic regions in Europe and North Africa*

The subject of Dr. Popiova's investigations includes the different chemical composition of propolis from subtropical climate zone in comparison to that of samples from temperate regions. She has studied chemical composition, antioxidant and antimicrobial activity of thirty two propolis samples from the Mediterranean region (Greece, Cyprus, Croatia and Algeria) and more than 150 components were identified by GC/MS, amongst which markers for *C. sempervirens* and *P. nigra*. The obtained

results confirmed that the change from temperate to Mediterranean climate results in changes in propolis chemistry - from poplar to diterpene type.

She also has identified the samples rich in phenolic acids and flavonoids which exhibited higher hypoglycemic and antioxidant activity and diterpene-rich samples with the highest antibacterial activity against *S. aureus, S. epidermis* and *S. mutans*.

*Propolis from tropical regions* (*from Asia, South Pacific islands, Africa, Asia* and *South America*

The tropical flora influence on the chemically variable composition of propolis from different tropical regions was confirmed by Dr. Popova‘s analyses of the GC/MS profiling of 8 samples from the Omani propolis. She has identified different compounds from the known propolis types and presents their significant chemical diversity.

For the samples from *Acacia nilotica* and *T. laeviceps,* widespread in Oman, higher antibacterial activity against *S. aureus* and *E. coli* was established than that of Bulgarian propolis. The antibacterial activity against *Listeria monocytogene* and *Streptococcus pyogenes* was also determined for all of the isolated compounds from propolis from the honey bees.

For the first time a new information on propolis was provided from different sources such as the stingless bee *Lisotrigona cacciae*, Vietnamese L. cacciae and bees from different regions as South Pacific islands, from Africa, South America and East Asia. The chemical composition of propolis from different regions as well as from different crude ethanol extracts show that they are rich of bioactive compounds and several new compounds were identified by Dr. Popova.

Most of identified compounds from different regions of Columbia demonstrated high activity against *S. aureus* and *C. albicans*, with garcinoic acid being the most active compound.

***The second subarea*: An overview of propolis data**

Over 300 components have been found represented predominantly by esters of aromatic acids, terpenes as volatiles, aromatic acids and flavonoids. The available data about the chemical composition of propolis volatile plant sources, innovative phytochemical approaches for its investigation, biological activity against bee pathogens, as well as problems with its standardization were summarized and presented in review articles published in scientific journals and books.

The data about propolis application in new and promising areas and innovative products are summarized for the first time. The data show that propolis has a great potential for the improvement of performance of lifestock, food preservatives, food packaging, textiles for medical applications, etc

The main methods in propolis research are also summarized as propolis extraction, GC/MS analysis, strategies for characterization of different propolis types by GC/MS, validated methods for qualification of poplar propolis and *Taiwanese green* propolis as well as criteria for poplar type quality control.

**Thematical area II. Studies on medicinal plants**

Scientific contributions to the area 2: Investigation of the chemical composition and biological activity of medicinal plants.

The information provided by Dr. Popova about chemical composition of Bulgarian celery (*Apium graveolens*), a known medicinal plant confirmed that it is a rich source of biologically active ingredients and can be useful for the pharmaceutical industry.

She presents new natural compounds in extract, isolated from celery root, identified by GC/MS and/or NMR, and information about the total phenolic and total flavonoid content in celery leaves and roots collected from 19 locations in Bulgaria. Moreover, nineteen components with two novel iridoid glycosides have been extracted from root cultures of *Verbascum eriophorum* with potential to regulate the increased T-cell a characteristic for some pathologies, such as arthritis.

The obtained results provide information on the total phenolic content of the extracts of roots and areal parts of the medicinal plant *Geum urbanum* L. widespread in Bulgaria, which have been used in folk medicine for gastrointestinal disorders, gynecological diseases, hemorrhoids, etc.

*Plan for future work*

Dr. Popova will focus her future work on the field of laboratory ”Natural Product Chemistry“ and related to her scientific interests and competence in studies on chemical composition, plant sources and biological activity of propolis from different geographic regions and mainly on propolis from unexplored regions, and stingless bee species.

Using modern techniques and methods, studies on propolis, medicinal and aromatic plants, mushrooms, as well as research on waste will continue to be main research topic in her work as a part of the programme of the Centre of Competence.

**4. Assessment of the applicant's personal contribution**

Although many of the works of Assoc. Prof. Popova have been published in a collaboration with other authors, her role is obvious.

**5. Critical comments and recommendations**

I have no critical comments on the materials presented and the research. I highly recommend Dr. Popova to continue her studies on the bioactive compounds from different sources mainly studying the chemical composition and biological activity of medicinal and economically important plants.

**6. Personal impressions**

I know Assoc. Prof. Popova and my personal impressions are that she is a very able-bodied, purposeful and dedicated scientist, which has undoubtedly led to significant achievements, both learned and practical.

**CONCLUSION**

The presented results show that Assoc. Prof. Dr. Milena Petkova Popova has in-depth theoretical knowledge and professional skills in the scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances", demonstrating qualities and skills for conducting research and obtaining original and significant scientific contributions.

The research has been carried out using modern methodology and instrumentation. The obtained results are published in high ranked peer reviewed journals in the fields of chemistry and biochemistry, and greatly contribute to fundamental science and practice. The papers are cited hundreds of times in the world literature.

Dr. Popova fully satisfies the requirements of the Law for Scientific Development in the Republic of Bulgaria, the Rules of its Application as well as of the Internal Rules and Regulations of the Institute Organic Chemistry with Center for Phytochemistry for acquiring the academic position of "Professor".

Because of that, I am giving my positive assessment of her research, obtained results and scientific contributions and I propose to the Honorable Scientific Jury to award the academic position "Professor" to Assoc. Prof. Dr. Milena Petkova Popova in the field of in the field of 4.2. Chemical Sciences; 01.05.10 "Bioorganic chemistry, chemistry of natural and physiologically active substances".

10 September 2019

Sofia

Reviewer:

(Prof. Pavlina Dolashka, DSc)