**REVIEW**

**by PhD Ilina Nikolaeva Krasteva – professor**
of the materials, presented for participation in a competition
to occupy the academic position of „Professor"
**at the Institute of Organic Chemistry with Centre of Phytochemistry (IOCCP), BAS,**
in higher education area 4. Natural Sciences, Mathematics and Informatics, professional field 4.2 Chemical Sciences, scientific specialty Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Compounds

in the competition for the academic position "Professor", announced in The State Gazette, issue 43 of May 31, 2019 and on the website of IOCCP, BAS, for the needs of Lab. "Chemistry of Natural Compounds" as a sole candidate, participates:

**Assoc. Prof. Dr. Milena Petkova Popova**
from the Laboratory of Chemistry of Natural Compounds, IOCCP, BAS.

## 1. General presentation of the received materials

The set of materials presented in paper and electronic form, presented by Assoc. Prof. Milena Popova, is in accordance with the Regulations of the Development of the Academic staff of the Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences (IOCCP-BAS), and meets the criteria of the Institute for occupying the academic position "professor".

In order to participate in the competition, the candidate assoc. prof. Popova applied 35 scientific works, 33 of which were printed in refereed and indexed journals, and 2 were book chapters. The submitted works, 8 on indicator B and 27, on indicator G, as well as one registered utility model are accepted for review and reporting in the final evaluation. The list of citations in refereed and indexed journals and collective volumes not presented in other competitions, as well as participation in 17 research projects are also evaluated. A total of 42 scientific papers (presented in the general "List of publications") included in the dissertation for "Doctor" and for the acquisition of the academic position "Associate Professor" are not reviewed. The distribution of scientific papers in the competition according to the relevant Q factors is as follows: 11 in journals with Q1, 15 with Q2, 3 with Q3 and 4 with Q4. Lists and supporting documents for participation in scientific forums, implementation of contracts with foreign and national companies, specializations, scientific-organizational, educational and expert activity, reflecting the overall activity of the candidate, are presented.

## 2. Brief biographical information

Assoc. prof. Milena Popova was born on September 25, 1974 in Byala, Ruse District. She received her secondary education at the School of Industrial Chemistry "Prof. Dimitar Balarev", Ruse. In 1998 she completed her higher education at the Faculty of Chemistry at Sofia University as a Master of Chemistry and Physics, with a professional qualification as a teacher of chemistry and physics. The following year, she joined the IOCCP -BAS as a chemist, and in the period 2001 - 2014 she held consecutive academic positions as a research associate III degree, senior and chief assistant professor. In 2004, she obtained her PhD in Phytochemical Research on Propolis. She habilitated in 2014, and since September 2018 has been head of the Lab.

"Chemistry of Natural Compounds". She has conducted 4 specializations abroad, one of which is long-term (8 months) in the Laboratory of Pharmacognosy and Chemistry of Natural Substances at the Faculty of Pharmacy, University of Athens, Greece.

**3. General characteristics of the candidate's activity**

*Evaluation of scientific and scientifically applied activities*

The applicant's overall research activity is in the field of the announced competition (Bioorganic chemistry, chemistry of natural and physiologically active substances). In the competition for "Professor" assoc. prof. Popova participates with 35 scientific works, as follows: 32 have been published in journals with Impact Factor (Scopus and Web of Sciences), one in an internationally specialised journal and two in book chapters. The total impact factor is 59.412. Eight items are presented under Indicator C, with the corresponding number of points being 149, with a requirement of 100. Under Indicator D, the total number of points from 25 publications, two book chapters and one utility model is 572 (with a requirement of 250), under Indicator E 2344 (required minimum 200) and Indicator F – 317 (required minimum 150). The total score on the indicators significantly exceeds the required minimum for obtaining the scientific title of professor. All scientific papers are in English and most of them have been published in prestigious journals such as: Chem. Cent. J., Curr. Pharm. Biotechnol., PLoS One, RSC Advances, Micropor. Mesopor. Mat., Phytochemistry, Food Chem. Toxicol., etc. The personal participation of the candidate in the submitted scientific production is indisputable – in 8 publications she is the first author, in 10 is the second author, and in 2 – the author for correspondence.

Significant participation in national and international fora should be noted in scientific output - a total of 58. She has presented 2 plenary lectures and a lecture at the Vietnam Academy of Science and Technology in Hanoi, and the rest with posters.

The research activity of Assoc. Prof. Popova was funded by 17 projects: 9, under the auspices of the NSF, MES and 8 international ones. She was the manager of two national projects, with the raised funds being 235 000 BGN.

Participations in a number of contracts with foreign and national companies, institutions, universities and individuals are highly appreciated, which is indicative of the practical application of the studies conducted on the qualification of propolis.

In connection with the implementation of projects Dr. Popova has conducted four specializations abroad: Institute of Biomolecular Chemistry, Naples, Italy; Faculty of Pharmacy, University of Athens, Greece; University "St. Cyril and Methodius", Faculty of Science and Mathematics, Skopje, Macedonia and Research Laboratory at Phycosource, Paris, France.

*Evaluation of educational and pedagogical activity*

Assoc. prof. Popova was a scientific advisor to a doctoral dissertation on the topic: "Chemical composition and biological activity of propolis from different geographical regions ", successfully defended in 2006 by Boryana Trusheva.

The candidate has participated as a scientific adviser and consultant in the development of six diploma works of students from the Faculty of Chemistry and the Faculty of Chemistry and Pharmacy, Sofia University.

*Contributions (scientific and/or applied) and citations*

Dr. Popova has a clearly outlined profile of her research activities. According to presented author's inquiry, the scientific contributions from the overall research studies are summarized in two main directions:

1. Investigation of propolis from different geographic regions and bee species.
2. Investigation of the chemical composition and biological activity of medicinal plants.

Propolis analysis is the main part of the scientific work of Assoc. Prof. Popova. They are related to clarifying the chemical composition, plant sources and biological activity of propolis from different temperate and geographic zones and bee species. The main purpose of the phytochemical research is the attempt to create a database of mass spectra of isolated compounds as trimethylsilyl ethers (derivatized products) with a view to further dereplication of already known propolis types by GC/MS, a method mainly used in analyses.

The research of propolis from temperate regions (Europe, North America, Argentina, Southern Africa, Asia and New Zealand) determined that it is characterised by similar chemical composition with main biologically active components being phenolics (flavonoids, phenolic acids and their esters) originating from the resinous buds secretions of *Populus* spp. (Publ.№ **45, 49, 54, 62, 64, 65, 67).**

Studies on the chemical composition of Bulgarian propolis show that it is a high quality product, according to the standards approved by the International Honey Commission. The criteria proposed can be used for the standardization of Bulgarian propolis with a view to introducing of reliable and scientifically based ISO standards (№ **62)**.

A detailed chemical profiling of propolis from Poland, exhibiting antitumor and antimicrobial activity, was performed. The chemical composition was analysed by GC/MS (after derivatization) allowing rapid and reliable dereplication (№ **60**). More than 80 compounds were identified and it was found that the sample is a poplar type propolis of dual botanical origin. Components typical for *P. nigra* were revealed, amongst which pinobanksin 3-*O*-acetate, phenethyl and pentenyl esters of caffeic acid. The presence of glycerol esters of phenolic acids, together with the relatively high content of *p*-coumaric acid and its esters provides an evidence for the contribution of aspen *P. tremula* L. as a second plant source (**№ 64**).

A study was conducted on a possible relationship between both propolis chemistry and bee colony health in respect to *V. destructor* infection (a mite causing varroosis in the honeybee *Apis mellifera* L. The chemical composition of propolis harvested in an apiary near Avignon, France, of resistant and non-resistant to *V. destructor* bee families regarding the balsam content and chemical profile was investigated. It was found that the propolis of infected hives was characterized by an increased content of balsam. Over 60 components were identified and the content of caffeic acid and its pentenyl esters was higher in the samples of healthy colonies (**№ 45**). However, no such response was observed in the colonies infected by the spore-forming bacteria *Paenibacillus larvae,* causing the disease American foulbrood. Propolis samples from healthy and infected families were analysed. The samples from the infected colonies were characterized by reduced content of balsam as well as lower content of ferulic acid and coniferyl benzoate (**№ 65**).

An efficient approach for preparation of aqueous colloidal solution of a well-characterised poplar type propolis (from Bulgaria) by encapsulation within copolymeric micelles was developed. *In vitro* propolis release of 100% at the end of the 52th hour was observed. The novel micellar form exhibited *in vitro* high cytotoxicity on leukemic cells. This is the first study on

novel delivery system based on copolymeric micelles encapsulating the full spectrum of propolis components with potential for parenteral administration in aqueous media and cancer therapies (№ **54**). A utility model was registered, the license of which has been granted to "Organik BG" Ltd. with a term of 30.09.2025. For the first time, silver modified nanoporous silica materials as a novel delivery system of propolis were prepared. The resulting materials improved the water solubility of the propolis and the activity against *S. aureus*, *E. coli* and *C. albicans* (№ **67**).

The quorum sensing inhibitory activity and the chemical composition of 10 propolis samples from different regions of America were studied. More than 60 components were identified by GC/MS that led to classification of the samples into three groups. One group was characterised by a relatively high content of aromatic acids (benzoic, cinnamic, *p*-coumaric and ferulic acids) and benzyl *p*-coumarate, typical for the poplar propolis originating from *P. tremuloides* (American aspen). *P. fremontii* was identified as the most probable source of the samples with high flavonoid content, and one of the sources of propolis from the third group. From a sample of North Carolina (a mixed propolis type) four compounds were isolated, from which new for propolis were ethyl ether of *p*-coumaric alcohol and 6*β*-hydroxy betulonic acid. For the samples of *P. fremontii* as well as for flavonoid pinocembrin, quorum sensing inhibitory activity was found against *Cromobacterium violaceum* (№ **49**).

Studies of propolis **from subtropical climatic regions** have been conducted (Publ. №**51, 52, 70, 72**). Thirty two propolis samples from Greece, Cyprus, Croatia and Algeria were analysed in respect to their chemical composition, antioxidant and antimicrobial activity. More than 150 components were identified, amongst which markers for *C. sempervirens* and *P. nigra*. The main and/or the only components in 15 of the samples were diterpenes, whereas other, collected from Central and Northern Greece, Algeria and Croatia were characterised by the presence of flavonoids and phenolic acids esters. Sugars were the main components found for five of the samples. The highest antibacterial activity against *S. aureus*, *S. epidermis* and *S. mutans* was established for diterpene-rich samples (№ **52**).

The chemical composition, antioxidant and hypoglycaemic activity of 5 samples propolis from North and Northwest Morocco were analysed. Over sixty individual compounds were identified. Two of the samples belong to the poplar propolis type and other was of a mixed propolis type, poplar - Mediterranean. Diterpenes were found in Moroccan propolis for the first time. The samples rich in phenolic acids and flavonoids exhibited higher hypoglycaemic and antioxidant activity (№ **51**), while sample with lower content of secondary metabolites (rich in sugars) did not display antioxidant or antibacterial activity (№ **72**). For the first time, poplar type propolis was found to enhance the oxidative stability of oil/water emulsions (№ **70**).

The investigations of the Propolis **from tropical regions are presented** in publications №**43, 50, 53, 57, 59, 61, 63, 69, 74, 76** and **77**. Propolis from Oman (Southwest Asia) was studied for the first time. The GC/MS profiling of 8 samples revealed that the Omani propolis is different from the known propolis types and demonstrates significant chemical diversity. New for the propolis compounds were isolated and identified such as: 7-*O*-methyl-8-prenylnaringenin and 3',8-diprenylnaringenin and on 8-prenyl-5,7-dihydroxy-3'-(3-hydroxy-3-methylbutyl)-4'methoxy flavanone, identified by mass spectral fragmentation. The samples analyzed showed activity against *S. aureus* and *E. coli* higher than that of Bulgarian propolis. The highest activity was demonstrated from samples rich in prenylated flavanones (№ **43**).

A chemical analysis of propolis by from Thailand, Southeast Asia, obtained from *A. mellifera* and two stingless bee species *Tetragonula laeviceps* and *Tetragonula melanoleuca* was performed. Propolis from the honey bees displayed similar GC/MS profile, but completely

different from that of the stingless bees. From one of the samples, exhibiting high antibacterial activity, 5 cycloartane triterpenes, along with mixtures of anacardic acids, cardanols and cardols were isolated (№ **63**). A similar chemical composition was established for propolis from *T. laeviceps*, but different from that of *T. melanoleuca.* Six prenylated xanthones, along with dammarane type triterpene and furofurane lignan were isolated from *T. laeviceps* propolis. Eight triterpenes were isolated from propolis of *T. melanoleuca*, along with 4 hydroxytriterpenic acids identified by mass spectral fragmentation. Most of the compounds were new for propolis. This was the first report for isolation of xanthones from propolis and the first study of propolis from *T. melanoleuca*.

A propolis from the stingless bee *Lisotrigona cacciae* was studied for the first time. From a propolis sample of Vietnamese *L. cacciae* 18 compounds (phenols and triterpenes), including a mixture of resorcinols were isolated and identified. Six of them (flavane, homoisoflavanes and *O*-geranyl xanthones) were new propolis compounds. By using the isolated compounds as reference substances as well as literature data, detailed identification of 33 components as trimethylsilyl ethers was achieved. The xanthone α-mangostin and flavane 7,4'-dihydroxy-5-methoxy-8-methylflavane inhibited *S. aureus*, while homoisoflavane 10,11-dihydroxydracaenone was the only one displaying antiradical ability (№ **77**).

For propolis from Fiji islands, a GC/MS profile similar to that of propolis from the Pacific region was found. Six components were isolated: 2 new for propolis sesquiterpenyl stilbenes, 2 prenylated flavonols, one of which new for propolis, flavonol and triterpenic acid **(№ 53).** Four new cycloartane triterpenes (hydroxy and acetoxy cycloartenones), along with 17 known compounds were isolated from extract of Pitcairn propolis exhibiting antimicrobial and potential cytotoxic activity. For the new triterpenes, activity against *S. aureus, E. coli* and *C. albicans* was detected (№ **69**).

The chemical composition of propolis from Cameroon was analysed. Seven triterpenes were isolated, incl. new natural compounds methyl-3β,27-dihydroxycycloart-24-en-26-oate and 1′-O-eicosanyl glycerol (№ **59**). Known triterpenes and a fatty acid ester were obtained from acetone extract of a sample from North-West region exhibiting anti-ulcer activity (№ **57**).

The chemical composition of propolis from different regions of Colombia was analysed. The high relative content of benzophenones, mainly nemorosone, resulted in higher antiradical activity and cytotoxic action against osteosarcoma cells (**№ 74**). From a sample from Val three $\delta$-tocotrienols were isolated: 2 new natural compounds (5,6-dioxo garcinoic acid and 5-hydroxy-8b-oxo garcinoic acid), along with known derivative garcinoic acid (*trans*-13'-carboxy-$\delta$-tocotrienol). All three derivatives belong to a rare class of compounds and are the first representatives of tocotrienols with oxidized terminal chain found in propolis. The isolated compounds as well as the crude ethanol extract demonstrated high activity against *S. aureus* and *C. albicans* **(№ 76).**

Essential oils' chemical characterization of two distinct propolis types, Brazilian red (plant source *Dalbergia ecastophyllum*) and Taiwanese green (*Macaranga tanarius*) propolis was performed. It was found that the main essential oils' components of the red propolis were phenylpropanoids, whereas in the green one oxidised sesquiterpenes, aliphatic alcohols, aldehydes, ketones, and hydrocarbons were characterised. This was the first report on the essential oil composition of Taiwanese green propolis (**№ 61**).

An overview of data on propolis is presented in review articles published in scientific journals and books (Publ. № **44, 47, 55, 56, 60, 71, 73**).

Another area in which Dr. Popova works is investigation of chemical composition and biological activity of four plants: *Apium graveolens, A. nodiflorum* (Publ. № **46, 48**), *Verbascum eriophorum, V. nobile* (Publ. № 58, 68) and *Geum urbanum* (Publ. № **66, 75**). From root extract of *A. graveolens* phthalides, allylphenoxyacetate, fatty acids, hydrocarbons, and coumarins, one of which 6-(3'-methyl-1'-oxobutyl)-7-hydroxycoumarin is a new natural compound, were isolated and identified by GC/MS and/or NMR. Total phenolic and total flavonoid content in celery leaves and roots collected from 19 locations in Bulgaria, including some wild-growing *A. nodiflorum*, were also analysed (**№ 46**). For methanol extract of *A. nodiflorum*, inhibition of the development of experimental osteoporosis in rats was found (**№ 48**).

The metabolic differences between wild plant and hairy root cultures of *V. eriophorum* were studied by NMR metabolomic and chemometric tools. Iridoid glycosides (harpagoside, aucubin and its derivatives) were observed only in the wild plant, whereas the *in vitro* cultures were found to be rich in phenylethanoid glycosides (**№ 58**). Two novel iridoid glycosides (*E*)- and (*Z*)-*p*-coumaroyl-3"-menthiafoloyl-rhamnopyranosylcatalopol, along with 9 known components were isolated. It has been found that the novel components have the potential to regulate the increased T cell activation characteristic for some pathology, such as arthritis (**№ 68**).

The first study on the chemical composition-biological (antimicrobial and anti-radicidal) activity of root and areal parts of *G. urbanum* L. was performed. From the ethyl acetate extract of roots, exhibiting the highest antimicrobial (against Gram (+) bacteria) and anti-radical activity, 7 components were isolated, among which two were found for the first time in the genus *Geum*, and three others - were newly detected for the species. For the flavane catechin, high activity against *S. aureus* and *C. albicans* (**№ 66**) was found, along with quorums sensing inhibitory potential against *Pseudomonas aeruginosa* for the root ethyl acetate extract (**№ 75**).

The results of the applicant's research activities have found a significant positive response among the Collegium of specialists in the field. The total number of **citations** (after the competition for Assoc. Prof.) is significant – 1529 citations of which: 1172 – in refereed and indexed journals and collective volumes (Web of Science and/or Scopus), 224 – in non-refereed journals and books and 133 – in dissertations. This data is admittedly evidence of the high scientific value of the published results.

*Implementation activities*

In collaboration with the Institute of Polymers - BAS, a utility model "Composition of water-soluble form of propolis" was registered, certificate № 2260 dated 07.09.2016. The license was granted to Organic BG EOOD until 30.09.2025.

*Scientific, organisational and expert activity*

Dr. Popova is a member of the editorial board of the Austin Journal of Bioorganic and Organic Chemistry and a guest editor of Natural Product Communication. In 2015, she received a team award – "SIB ITI 2015" trophy for the development of "Propolis Water Soluble Composition".

Her expertise is mainly related to peer review of publications in a number of international journals. She has participated in a committee for awarding the educational and scientific degree "doctor" to doctoral student Svetoslav Alexandrov, Institute of Plant Physiology and Genetics – BAS.

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

The personal contribution of Dr. Popova in the presented scientific production is undeniable. It presents proven, original and significant scientific contributions, especially in the field of chemistry of natural compounds, with theoretical developments being of practical use.

**5. Critical remarks and recommendations**

In essence, I have no critical remarks. My recommendations to Assoc. prof. Popova are to expand and share her experience and knowledge by teaching more students and PhD students.

**6. Personal impressions**

My personal impressions of the candidate are that she is an experienced and knowledgeable researcher, with ideas and prospects for development, with a wide range of interests and high competence in the field of phytochemistry and chemistry of natural compounds.

**CONCLUSION**

The only candidate in the PROFESSOR competition announced for the needs of the lab. "Chemistry of Natural Compounds" of the Institute of Organic Chemistry with the Center of Phytochemistry, BAS, participates in the competition convincingly with sufficient scientific and scientific-applied production, as well as with its entire research and expert activity. Undoubtedly, the results achieved by assoc. prof. Milena Popova are in full compliance with the specific requirements of the IOCCP -BAS and the implementation of the Regulations of the Development of the Academic staff.

Dr. Popova is a specialist with a clear scientific profile. The high qualification and research activity is undoubtedly confirmed by her participation in a large number of interdisciplinary research and scientific projects.

The analysis of the submitted scientific papers, their scientific and applied scientific contributions and scientific appearances, as well as the personal qualities of the candidate give me reason to persuade the members of the Distinguished Scientific Jury to vote positively for the election of assoc. prof. Dr. Milena Petkova Popova for the academic position of “Professor” at IOCCP-BAS in the professional field 4.2 Chemical Sciences, scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active compounds".

9 September 2019 Reviewer:

/Prof. Dr. I. Krasteva/