REVIEWS

for the competition for the academic position "Professor"

in professional field 4.2. Chemical sciences, "Bioorganic Chemistry, Chemistry of natural and physiologically active substances" for the needs of Laboratory for Natural Chemistry, Institute of Organic chemistry with Centre of Phytochemistry – BAS

Applicant: Assoc. Prof., PhD Antoaneta Borissova Trendafilova-Savkova

Reviewer: Prof., PhD Svetlana Temelkova Bancheva, Institute of Biodiversity and Ecosystem Research – BAS

In the announced competition for the occupation of the academic position "Professor" in the professional field 4.2. Chemical Sciences, "Bioorganic chemistry, chemistry of natural and physiologically active substances" for the needs of Laboratory for Natural Chemistry, Institute of Organic chemistry with Centre of Phytochemistry – BAS, one candidat participated – Dr. Antoaneta Borisova Trendafilova-Savkova, Assist. Prof. at the same Laboratory. I have no joint publications with the applicant submitted for participation in the competition. The documents presented by Assoc. Prof. Trendafilova-Savkova show that the procedure for its announcement is in order and they are in accordance with the requirements of the Law for the development of the academic staff in the Republic of Bulgaria and the Rules for its implementation, as well as with the Rules and Conditions for the acquisition of academic degrees and for the occupation of academic positions in BAS and the Institute of Organic chemistry with Centre of Phytochemistry (IOCCF).

1. General data on the career and thematic development of the applicant

Antoaneta Trendafilova-Savkova completed her secondary education in 1986 at the Model School of Chemical Industry and Biotechnology "Prof. Dr. Assen Zlatarov "Sofia. In 1991 she obtained a master's degree from the Sofia University "St. Cl. Ohridski ", Faculty of Chemistry, with master degree in Organic and Analytical Chemistry. One year later she was appointed as a specialist chemist at IOCCF-BAS. In the period 1995-1997 he developed a PhD thesis in the scientific area "Bioorganic chemistry, chemistry of natural and physiologically active substances". Her scientific career continued at the same institute, being

a associate researcher until 2011. After successfully winning the competition in the same year she was appointed to the position of Assoc. Prof.

The main scientific interests of Assoc. Prof. Trendafilova-Savkova are in the field of chemistry of natural compounds, and in particular - phytochemical studies of aromatic, medicinal, endemic and *in vitro* grown plants - extraction, isolation and structural characterization of secondary metabolites (essential oils, terpenoids and phenolic compounds).

The total number of publications of the candidate during her entire scientific career are 80, the citations of works with her participation are over 350, and her h-index is 11. The list of publications that do not participate in the competitions for the acquisition of her PhD degree and the academic position "Associate Professor" includes 51 titles.

In this competition Assoc. Prof. Trendafilova-Savkova participates with 33 scientific works, which in accordance with the national requirements are divided into 2 groups: *by indicator C – 13 (Q1 - 3, Q2 - 8, Q3 - 2); by indicator D – 20 (Q1 - 5, Q2 - 4, Q3 - 7, Q4- 4).* The applicant's report shows that it fully covers, and in most indicators (C, D, E and F) even exceeds the national minimum requirements for the academic position „Professor".

2. Main areas of the applicant's research and the most important scientific contributions

Assoc. Prof. A. Trendafilova-Savkova has been working in several very promising areas that humanity has paid much attention to in recent years, such as the use of natural substances as nutritional supplements or as alternative ways of prevention and treatment. The most important of these are:

- Phytochemical investigations of medicinal plants, endemics and/or unexplored taxa aimed at obtaining new data on the structural diversity of biologically active components, their biological activity and their influence on biological objects.

  These are studies related mainly to isolation and structure elucidation of sesqui-, di- and triterpenoids, flavonoids, coumarins, furanocoumarins and phenolic acids from plants of different plant families such as Asteraceae, Rosaceae, Apiaceae and Araceae. In my opinion, a significant contribution of the applicant is the isolation of over 140 individual compounds using modern chromatographic techniques, of which 24 are newly discovered natural compounds identified by spectral methods. I think it is worthwhile to draw attention to the feature of the study on the chloroform

extract obtained from the aerial parts of *Artemisia alba*, in which 13 individual compounds have been isolated – 11 sesquiterpenoids and 2 flavonoids. Three of them have been found for the first time in the investigated species and another 10 were new natural compounds – biogenetically related sesquiterpenoids with a germacrane, a eudesmane, a guaiane and an oplopane carbon skeleton and with a high degree of oxidation. The methanol extract of *A. alba* afforded 18 compounds, which have been identified as follows: 3 esters of quinic acid, 2 coumarins and 13 flavonoids. Five of them have been reported for the first time in the investigated species. A comparison of *A. alba*'s flavonoid profile with literature data has shown that the samples from the Balkan Peninsula produced both flavones and flavonol types of compounds in contrary to those from the Mediterranean countries, which contained only flavonol type.

From the aerial parts of *Inula aschersoniana* 8 compounds have been isolated: 3 sesquiterpene lactones, a flavonoid and 4 new pseudoguaiane-type sesquiterpenoids. The new compounds contained a cyclopropenone ring in their molecule. These types of compounds are rare in natural sources.

It has been found a significant difference in the skeleton type of sesquiterpene lactones accumulated in European and Asian origins of the species. Thus, the domination of guaianolides and eudesmanolides or their seco-derivatives characterize European and Asian taxa, respectively.

From the chloroform extract obtained from the aerial parts of *I. oculus-christi* 6 newly discovered sesquiterpene lactones have been isolated (1 eudesmanolide and 5 guaianolides). The results may also have chemotaxonomic use. From the methanol extract obtained from the flowers 6 flavones, 2 flavone glucosides, chlorogenic acid and 3,5-dicaffeoylquinic acid have been isolated. With the exception of hispidulin, all identified compounds are detected for the first time. Preliminary cytotoxicity study of extracts and fractions enriched in sesquiterpene lactones, flavonoids and phenolic acids from *I. oculus-christi* has shown stronger impacts of studied extracts on cancer cells rather than on non-cancer cells.

The Balkan endemic species *Jurinea tzar-ferdinandii* has been studied for the first time, from which 22 components have been isolated and identified: 12 triterpenoids, 6 sesquiterpene lactones and 4 flavones. The isolated sesquiterpene lactones have germacrane, eudesmane and elemane carbon skeleton and are first isolated from a representative of genus *Jurinea*. The lack of any functional group

at C-14 distinguished them from the lactones found so far in the genus *Jurinea*, all of which contained oxygenated substituent at this position. All isolated flavonoids are 5,7-dihydroxy-6-methoxyflavones, differing in the substitution pattern of ring B: 4'-mono- and 3',4'-disubstituted with OH and/or OMe groups. The presence of flavonoids in *Jurinea* species supports biogenetic relationship of genus *Jurinea* with the genera *Centaurea* и *Onopordon*.

For the first time the endemic species *Anthemis rumelica* has been studied and from its flower heads have been isolated and structurally characterized 2 flavonoids and 4 sesquiterpene lactones, two of which are new natural compounds with a guaiane carbon skeleton.

The plant species *Asterothamnus centrali-asiaticus* from the Gobi Desert (Mongolia) has been studied phytochemicaly for the first time. A new cembrane glycoside has been isolated from the aerial parts of the species which is the first C-15 analog of (–)-nephthenol isolated from vascular plant.

Three endemic species of the genus *Alchemilla* have been investigated, for which data on their chemical composition are missing: *A. mollis* (critically endangered species in Bulgaria) and Bulgarian endemics - *A. achtarowii* (endangered) and *A. jumrukczalica* (critically endangered). A total of 24 flavonoid glycosides, mainly of kaempferol and quercetin types, have been isolated. A new compound has been found, galactopyranosyl-7-O-α-L-rhamnopyranoside that showed relative good antiradical scavenging activity.

For the first time the contents of furanocoumarin content, antioxidant potential and acetylcholinesterase and *α*-amylase inhibitory activities of hexane extracts of four *Heracleum* species have been investigated by the applicant. The leaf and fruit extracts of *H. angustisectum* has shown the highest DPPH radical scavenging activity and TEAC, while the roots extracts of *H. verticillatum* and *H.angustisectum* – the most effective inhibitors of acetylcholinesterase.

The chromatographic separation of the butanol fraction obtained from aqueous-methanolic extract of the medicinal plant *Arum palaestinum* from Jordan led to isolation of 5 flavonoid glycosides and two of them are reported for the first time in the investigated species.

- Investigation of essential oil composition of aromatic and medicinal plants and identifying the main components in by using GC and GC-MS aimed at using them as chemotaxonomic markers.

  The composition of 9 species of the Asteraceae, Lamiaceae и Apiaceae families has been studied. More than 200 components have been identified by using GC and GC-MS. The identification of the compounds has been performed by comparison of their mass-spectral characteristics and retention times with those published in the literature. As a result of these studies, knowledge of the essential-oil profiles of the species studied has been enriched, some correlations between chemical composition and environmental conditions have been identified, as well as their use as chemotaxonomic markers, and the influence of growth regulators on the accumulation of certain components in *in vitro* grown plants have been established.

- Quantitative determination of biologically active compounds in medicinal plants.

  These studies are important, not only from a fundamental but also from applied point of view, in order to study the factors affecting the secondary metabolism of medicinal plants, to evaluate the efficacy of attempts to cultivate them, to look for a link to the biological activity of extracts, chemotaxonomic relationships and and others. I'll address some of them: the content of sesquiterpene lactones and the lactone profile of *Arnica montana* in *in vitro* and *in vivo* propagated plants obtained by seeds of different origins and grown in two different experimental fields have been investigated; extracts from leaves and flowers of *A. alba*, *I. britannica* and *I. oculus-christi* have been studied for their total phenolic and flavonoid content as well as their antioxidant capacity against DPPH˙and $ABTS^{\cdot+}$ using spectrophotometric methods; the effectiveness of *ex vitro* adaptation, acclimatization and cultivation of 3 endemic *Alchemilla* species in two different experimental fields has been evaluated by spectrophotometric determination of flavonoids and tannins; the amount of furanocoumarins in the different parts of 4 *Heracleum* species has been investigated and the roots and fruits found to be the richest in this class of natural compounds. These data can also be used in chemotaxonomy.

I would like to emphasize that in her research my colleague uses modern techniques for the extraction of biologically active substances. In addition, I fully accept the reference for the

scientific contributions submitted by the applicant. I find it objective and accurately reflecting the interdisciplinary research of Assoc. Prof. Trendafilova-Savkova.

3. Evidence of the significance of the studies conducted

Assoc. Rrof. Trendafilova-Savkova presented a list of 250 citations of works with her participation, which is evidence of the relevance of her research. Some of her publications have been cited in double-digit publications, such as Publication No. 30, which has 28 citations, Publication No. 36 - 25 citations, Publication No. 55 - 19, and so on. Also noteworthy is the participation of the candidate in scientific forums with poster or oral reports - a total of 63 participation in the post-habilitation period to date.

4. Training of students and PhD students

Assoc. Prof. Trendafilova-Savkova has participated in the leadership of 1 PhD student, 4 master students and 2 students in the "Student practices program". This is a evidence to her pedagogical skills and a willingness to pass on her experience to young researchers.

5. Participation in research projects and fundraising

Assoc. Prof. Trendafilova-Savkova has participated in the implementation of 21 research projects, 4 of which are international, funded by the EC Horizon 2020 program, the Bulgarian-Swiss Scientific Program and the Ministry of Foreign Affairs of Italy; 5 under equivalent non-cash exchange projects of BAS; 5 national, funded by the NSF and 5 funded by other sources. She has successfully managed 2 projects from the NSF and 2 under the equivalent non-cash exchange of BAS, providing a total of 144,000 BGN. All this gives me confidence that the applicant has the necessary skills and experience to organize and lead a research team, as well as to generate ideas and hypotheses, and successfully implement them.

6. Questions and Recommendations

I allow myself to recommend to my colleague in her future work, in her new position, to be a little more active in seeking opportunities for attracting research funds by developing new project proposals. In my opinion, it is also necessary to do what is necessary to attract young scientists and PhD students to join her team and continue her research in the scientific fields in which she is a specialist.

I have two questions:

- The attracted funds according to the inquiry amount to BGN 144 000. Has the number of participants in the projects been taken into consideration and the

amount allocated accordingly, or is this the total amount of the projects? In my opinion, the right approach is to allocate the amount to the participants in the projects.

- Does the colleague intend in the near future to prepare a new scientific project to lead and have a specific idea of its topic and scope?

7. Conclusion

On the basis of the materials presented in the competition by Assoc. Prof. AntoanetaTrendafilova-Savkova I am convinced that it fully meets and by some indicators far exceeds the national criteria for occupying the academic position “Professor“, defined by the Law for the Development of Academic Staff in the Republic of Bulgaria, The Rules for its implementation, the Rules for the conditions and the order for occupying academic positions in BAS, as well as the Rules for the conditions and the procedure for acquiring academic degrees and for occupying academic positions in the IOCCF-BAS. The applicant is an established scientist with a clearly defined scientific profile and proven scientific and applied contributions. The field in which she works is perspective and with great scientific and applied interest. My impressions are that Assoc. Prof. Trendafilova-Savkova is a very good professional, correct and desirable partner for scientific and applied scientific projects.

On the basis of the above, I strongly recommend the members of the Scientific Jury to support the selection of Assoc. Prof. Antoaneta Trendafilova-Savkova for the occupation of the academic position „Professor“ in the professional field 4.2. Chemical Sciences, „Bioorganic chemistry, chemistry of natural and physiologically active substances“.

08.09.2019 г. Reviewer:

Sofia (Prof. PhD S. Banchevs)