SCIENTIFIC APPRAISAL

of Prof. Dr Alexander Nikolov Tashev, University of Forestry – Sofia

on the documents presented by Assoc. Prof. Dr Antoaneta Borissova Trendafilova-Savkova, Institute of Organic Chemistry with the Center of Phytochemistry (IOCCP) –BAS for participation in the competition for the occupation of the academic position "Professor" at the IOCCP - BAS in the field of Chemical sciences, code 4.2. Scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active compounds" for the needs of the laboratory "Chemistry of natural products" declared in the State Gazette, issue 43 / 31.05.2019

1. Short bibliographic data of the applicant.

Antoaneta Borissova Trendafilova-Savkova was born on 31.10.1967. She was graduated in 1986 from the technical school for chemical industry and biotechnology "Prof. Dr Assen Zlatarov", Sofia. She was graduated in Organic and Analitycal Chemistry in 1991 from the Faculty Chemistry at Sofia University "St. Kliment Ohridski". She started working as a chemist-specialist in 1992 at the Institute of Organic Chemistry with the Centre for Phytochemistry, BAS, where she is currently working. From 1993 to 2011 she worked as an Assistant,. Assistant Professor, and from 01.10.2011 - to this day she is an Associate Professor at IOCCP, BAS, in the Laboratory "Chemistry of natural products". In the period 1995-1997 she is a doctoral student at IOCCP, BAS, where acquire an educational and scientific degree "PhD" in the scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active compounds".

2. General presentation of the presented materials

The applicant Antoaneta Borissova Trendafilova has presented only publications after acquiring the scientific title "Associate Professor". The presented materials can be classified as follows:

- publications outside competitions for acquisition of the scientific and educational degree "doctor" and the academic position "associate professor" – 51;
- publications under indicator V – 13 (Q1 - 3, Q2 - 8, Q3 - 2)
- - publications under indicator G – 20 (Q1 - 5, Q2 - 4, Q3 - 7, Q4- 4)

All presented publications are written in English. She is sole author of 1 publication and co-author of the other 50, which have one to 24 co-authors. In 16 publications the applicant is the first author, in 20 publications she is the second author, in 7 - the third author, etc. For the period from 2010 until now A. Trendafilova has participated in 63 scientific conferences and 21 research projects, 9 of which are international, 7 funded by the NSF and 5 funded by other sources. She has been the manager of 4 projects: 2 funded by the NSF and 2 bilateral international projects.

3. Reflection of the applicant's scientific publications in literature.

To date, according to data taken from Web of Science and Scopus, the applican**t** has found 250 citations to 72 of her publications for the periods beyond the competitions for the doctorate and academic degrees, i.e., only for the period 2010 to the present. Most of these quotes are in Impact Factor magazines. This fact testifies to the high scientific level of her publications.

4. General characteristics of the applicant's activities:

4.1. Educational and pedagogical activity.

The teaching and pedagogical activity of Assoc. Prof. Trendafilova consists in the guidance of trainees, graduates and doctoral students. She has led four graduate students at the Master's Degree Program – two from the Faculty of Chemistry at Sofia University „ St. Kliment Ohridski " and two from the University of Chemical Technology and Metallurgy. She was a scientific adviser to a doctoral student who successfully defended her dissertation. She has also directed two postgraduate students from the Medical University and the Faculty of Chemistry at Sofia University "St. Kliment Ohridski "in the frame of Student Practice Projects.

4.2. Scientific, applied and methodological activity:

The scientific achievments presented in the completion are result of interdisciplinary research, which could be summarized in the following directions:

- phytochemical investigations of medicinal plants and/or unexplored taxa so far to obtain new data on the structural diversity of biologically active components, chemosystematics, biological activity and mechanism of action on various biological objects;
- preparation of essential oils from aromatic and medicinal plants, gas chromatography and mass spectral analysis of essential oils with a view to identifying the their main components and searching for chemotaxonomic and other relations;
- use of modern techniques for the extraction of biologically active compounds from medicinal plants;
- quantitative determination of biologically active compounds in medicinal plants.

4.3. Scientific, applied and methodological contributios.

4.3.1. **Phytochemical investigations of medicinal plants and/or unexplored taxa.**

These investigations are connected mainly with 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. More than **140** individual compounds are isolated using modern chromatographic techniques, of which **24** were new natural compounds identified by spectral methods.

4.3.1.1. Secondary metabolites in the representatives of family Asteraceae:

- Thirteen compounds (11 sesquiterpenoids and 2 flavonoids) were isolated from the chloroform extract obtained from the aerial parts of ***Artemisia alba***. Three of them were found for the first time in the investigated species and another 10 are new natural compounds. The methanol extract of ***A. alba*** afforded **18** compounds, which were identified as follows: 3 esters of quinic acid, 2 coumarins and 13 flavonoids. The presence of **five** of isolated compounds is reported for the first time in *A. alba*;
- The subendemic species ***Inula aschersoniana* var. *aschersoniana*** was studied phytochemically for the first time. The aerial parts of *Inula aschersoniana* Janka var. *aschersoniana* afforded 8 compounds: 3 sesquiterpene lactones, a flavonoid and **4** new pseudoguaiane-type sesquiterpenoids;
- Alantolactone and isolanatolactone were isolated from the roots of elecampane (*Inula helenium* L.), for which various pharmacologic activities such as hepatoprotective, anti-inflammatory, antitumor, antibacterial, antidematophytic, antifungal activities, etc. has been reported;
- The flower heads of ***Inula britannica*** L. of Bulgarian origin afforded 5 sesquiterpene lactones with guaiane, pseudoguaiane and eudesmane carbon skeleton, 3 triterpenoids, 3 flavonoids and 1,5-dicaffeoylquinic acid;
- Investigation of the chloroform extract obtained from the aerial parts of ***Inula oculus-christi*** L. led to the isolation and identification of 6 new sesquiterpene lactones in addition to the known gaillardin, pulchellin E, pulchellin C and 3-O-palmitates of 16β-hydroxylupeol, 16β-hydroxy-β-amyrin, and faradiol. Six flavones, 2 flavone glucosides, chlorogenic acid and 3,5-dicaffeoylquinic acid were isolated from the

methanol extract, obtained from the flower heads of ***I. oculus-christi*** **L.** With exception of hispidulin, all identified compounds were detected for the first time in the studied species;

- The Balkan endemic species ***Jurinea tzar-ferdinandii*** **Davidov** was studied for the first time and led to the identification of 22 components;
- The inhibitory potential of the total chloroform extract, fractions containing triterpenes, flavonoids and sesquiterpene lactones as well as individual compounds against a bacterial lipase from *Candida rugosa* (CRL) and a lipase from porcine pancrease (PPL) was also evaluated;
- The endemic species ***Centaurea davidovii***, was studied for the first time and 8α-(5'-hydroxyangeloyl)-salonitenolide was isolated and identified;
- For the first time the endemic species ***Anthemis rumelica*** has been studied and from its flower heads were isolated and structurally characterized 2 flavonoids and 4 sesquiterpene lactones, **two** of which are new natural compounds with a guaiane carbon skeleton;
- ***Asterothamnus centrali-asiaticus*** Novopokr. collected from Gobi desert (Mongolia) was investigated for the first time. A new cembrane glycoside was isolated from the aerial parts of the species. The newly described compound is the first C-15 analog of (–)-nephthenol isolated from vascular plant up to now.

4.3.1.2. Secondary metabolites in representatives of genus *Alchemilla* (Rosaceae).

- The work-up of the EtOAc fraction obtained after partition of the total methanol extract from the aerial parts of the three *Alchemilla* species (*A. mollis a*nd the Bulgarian endemic *A. jumrukczalica* and *A. achtarowii)* led to isolation of 24 compounds in total, one of them was found to be a new natural compound;
- Antioxidant capacity of the methanol extract, fractions and individual compounds from ***A. mollis*** and ***A. jumrukczalica*** was measured by their ability to scavenge the DPPH radicals.

4.3.1.3. Secondary metabolites in species of genus *Heracleum* (Apiaceae).

- For the first time hexane extracts of four *Heracleum* species, growing in Bulgaria – Balkan endemic *H. verticillatum*, Bulgarian endemic *H. angustisectum*, *H. sibiricum*, and *H. ternatum* were studied for their furanocoumarin content, antioxidant potential and acetylcholinesterase and *α*-amylase inhibitory activities. The performed qualitative NMR analysis allowed identification of 9 furanocoumarins in the extracts from leaves, roots and fruit.

4.3.1.4. Secondary metabolites from *Arum palaestinum* (Araceae).

- The chromatographic separation of the butanol fraction obtained from aqueous-methanolic extract of the medicinal plant ***Arum palaestinum*** **(**originating from Jordan) led to isolation of 5 flavonoid glycosides and two of them are reported for the first time in the investigated species. The butanol fraction exhibited a significant activity against MCF7 and HepG2 cell lines, while aqueous-methanolic extract – significant antivirus activity against H5N1virus.

4.3.2. Investigation of essential oil composition of aromatic and medicinal plants and *in vitro* cultures.

The essential oil composition of nine species from families Asteraceae (*A. alba*, *Inula britannica*, *I. aschersoniana*, *I. oculus-christi*), Lamiaceae (*Sideritis scardica*, *Panzeria lanata* and *Thymus longedentatus*) and Apiaceae (*Seseli rhodopeum* and *S. rigidum*) was studied. More than 200 components were identified by using GC and GC-MS. The identification of the compounds was performed by comparison of their mass-spectral characteristics and retention times with those published in the literature. Some more significant contributions are presented below:

- The essential oil composition of six native populations of ***Sideritis scardica*** from Bulgaria (Pirin, Slavyanka and Rodope Mts) was studied. Altogether, 37 components were identified. Among them, α- and β-pinene, phenylacetaldehyde, β-bisabolene, benzyl benzoate, and m-camphorene were the main compounds;
- Volatile components of nine ***S. scardica*** samples from cultivar planted at different ecological conditions were studied. It was established a significant chemical polymorphism of the samples and a relation between the oil profiles, altitude and climatic zones, where the plants were grown up;
- The chemical composition of the volatile extract from aerial parts of ***Inula oculus-christi*** L. was studied by GC-MS for the first time;
- The separately distilled flowers and leaves essential oils of ***I. britannica*** L. were investigated and a total of 83 constituents were registered. The oils were rich in terpenoids;
- The study of the volatile oil of *Inula aschersoniana* was resulted in detection of 45 constituents. The oil contained fatty acids (55.2%) and alkanes (14.1%) and relatively low content of terpenoids (16.7%);
- Five essential oils obtained from ***Artemisia alba*** shoots were analyzed by GC and GC-MS. Comparison of the oil composition based on up to 34 components in concentrations exceeding 0.5% in at least one of the samples led to determination of the two main types of oil.

4.3.3. Comparison of different techniques for extraction of biologically active compounds from medicinal plants.

The effects of ethanol concentration, extraction time, temperature and number of extraction steps on the extraction yields of alantolactone and isoalantolactone in roots of ***I. helenium*** were investigated. A comparison with classical extraction methods [maceration, infusion and micro steam distillation-extraction (MSDE)] showed that the amounts of alantolactone and isoalantolactone achieved by UAE with 70 and 96% EtOH for 30 min at room temperature were higher or almost equal to those obtained by maceration for 24 hours.

4.3.4. **Quantitative determination of biologically active compounds in medicinal plants.**

These investigations are connected with the application of the classical spectrophotometric methods, chromatographic methods (GC, HPLC, etc.) and spectroscopic methods (NMR) for quantitative determination of main group of compounds (total phenolic content, total flavonoid content, tannins, etc.) and/or principal compounds in plant extracts. The results are used for quality control, to study of factors affecting the secondary metabolism of medicinal plants, for evaluation of efficacy of cultivation attempts, to search for a connection with the biological action of extracts, for chemotaxonomic correlations, etc. The results of this interdisciplinary research have not only scientific, but also **scientific and applied** contributions.

4.3.4.1. Sesquiterpene lactones in *Arnica montana.*

The amount of sesquiterpene lactones and the lactone profile of *A. montana* in flowering and seed formation stages *in vitro* and *in vivo* propagated from seeds of German, Ukrainian, and Austrian origin and grown in two experimental fields were studied. GC method was used for the quantification of sesquiterpene lactones.

4.3.4.2. Secondary metabolites in *Centaurea davidovii.*

The accumulation of phenolic compounds and flavonoids was determined spectrophotometrically and that of sesquiterpene lactone 8α-(5'-hydroxyangeloyl)-salonitenolide - by HPLC. The composition of the nutrient medium influenced the contents of all studied bioactive substances.

The obtained results revealed both the effectiveness of biotechnological methods for propagation and conservation of rare and endangered plant species, and the possibility to use

C. davidovii plants *ex vitro* acclimated to field conditions as a source of secondary metabolites with potential biological activity.

4.3.4.3. Total phenolic and flavonoid content in *Artemisia alba, Inula britannica and I. oculus-christi.*

- Extracts from leaves and flowers of *A. alba, I. britannica* and *I. oculus-christi* were studied for their total phenolic and flavonoid content as well as their antioxidant capacity against DPPH˙and ABTS˙+ using spectrophotometric methods.

4.3.4.4. Total flavonoid content and tannins in *Alchemilla* species.

- Comparison of extracts from *in vitro* cultivated *A. mollis* (different media and plant regulators), *ex vitro* adapted and acclimated plants showed the highest amount of flavonoids in the shoot cultivated on the control MS as well as in the *ex vitro* adapted plants. The highest tannin content was registered in the shoot cultivated on the ½ MSA and *ex vitro* adapted plants;
- The cultivation *ex situ* of *A. mollis, A. achtarowii* and *A. jumrukczalica* in two experimental high-mountainous fields (Vitosha and West Rodope Mt) could be succesful at an altitude similar to their natural distribution.

4.3.4.5. Furanocoumarins in *Heracleum* species.

- ^{1}H-NMR spectroscopy was used for qualitative and quantitative determination of furanocoumarins in 4 *Heracleum* species. The comparative 1H-NMR study of the hexane extracts of roots, leaves and fruits showed qualitative and quantitative differences between the organs of the plants as well as between the species. The fruits and roots extracts were rich in furanocoumarins. The similarity in the furanocoumarin profile of the Bulgarian endemic *H. angustisectum* and *H. ternatum* has been established. The chemical profile of the newly studied *H. angustisectum* was similar to that of *H. sibiricum* and *H. ternatum* and therefore could be placed it in the same group..

5. Assessment of the applicant's personal contribution.

Almost all scientific works, except for one, submitted by Assoc. Prof. Trendafilova for participation in the competition for professor in "Bioorganic chemistry, chemistry of natural and physiologically active compounds" in section 4.2. The chemical sciences are co-authored by two to 25 authors (from 1 to 24 co-authors), in 16 of them she is the first author. Probably, this can be considered a natural phenomenon due to the complex nature of the research in which she participated and demonstrated excellent teamwork skills and capabilities.

6. Critical remarks.

Some critical remarks can be made on the materials presented:

1. It is not clear in the submitted habilitation documents why the document is listed under number 2.2 in a folder named "18_AT_doctoranti_diplomanti" – It does not reveal the name of A. Trendafilova (Thesis: "Essential oil and flavonoid profile of *Artemisia alba* Turra ").
2. Contribution reports are too detailed, difficult to work with, and more like summaries of the abstracts of individual publications. It would be more sensible to present it in a more concentrated and synthesized way, focusing on scientific results that are new to the scientific field. There are also parts in this document that are not relevant to the candidate's contributions.
3. It is desirable that the applicants to have more independent publications in order to demonstrate their ability to work independently. The large number of co-authors in some publications (up to 24) may make it difficult to separate the applicant's personal contribution to those publications.

4. In the lists of publications and citations it would be good to indicate not only the quartiles (Q1-Q4) of Scopus and Web of Science journals, but also the details of scientific journals – Impact Factor, SJR, ISSN of scientific journals and ISBNs of books and monographs.
5. The proclamation of *Heracleum angustisectum* as an endemic species is not very correct because it has not been proven by appropriate studies. Rather, it is a leaf form of *Heracleum sibiricum*, which is found everywhere in Bulgaria. It is no coincidence that the candidate's phytochemical studies show the closeness of the two taxa.

7. Personal impressions.

My personal impressions of the candidate are related to our professional communication as colleagues in the field of phytochemistry. We have also had professional contacts in joint participation in international scientific forums. They show that the candidate has excellent communication skills, which is very important when working in a team, as well as a thorough knowledge of her scientific interests.

8. Conclusion.

The documents and materials, presented by Assoc. Prof. Dr. Antoaneta Borisova Trendafilova-Savkova, meet all the requirements of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the application of the ADASRB and the Regulations for the conditions and procedure for acquiring scientific degrees and for occupying academic positions at IOCCP-BAS and cover the requirements for the academic position of "Professor". Assoc. Prof. A. Trendafilova is an experienced leading researcher with a clearly defined scientific subject, which gives me reason to give my positive assessment and and I strongly recommend that the Scientific Board of IOCCP-BAS award to Assoc. Prof. A. Trendafilova the academic position of "Professor" in the professional field 4.2. Chemical Sciences, scientific specialty "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Compounds".

Sofia, September 10, 2019 Prepared the appraisal

/Prof. Dr A. Tashev/