

REVIEW

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on the materials submitted for participation in the competition for the academic position of Associate Professor at the Institute of Organic Chemistry with Centre for Phytochemistry (IOCCP), BAS in the field of higher education 4. Natural sciences, mathematics and informatics, professional field - 4.2 Chemical Sciences, scientific specialty - Bioorganic chemistry, chemistry of natural and physiologically active substances

1. General presentation of the materials received

In the competition for 'Associate Professor', announced in the State Gazette, no. 102 of 08.12.2023 and on the website of the IOCCP-BAS, Dr. Tsvetelina Emilova Doncheva, Assistant Professor at IOCCP, BAS is the only candidate. The set of paper materials presented by Dr. Tsvetelina Doncheva is in accordance with the Rules for the Development of the Academic Staff of the IOCCP and meets the criteria of the IOCCP-BAS for occupying the academic position "Associate Professor". A report on the fulfillment of the minimum requirements, a report on scientific contributions, a list and copies of scientific works on the competition (a total of 19 items, corresponding to the scientific specialty of the competition), as well as an abstract for the PhD acquisition and lists of participation of the candidate in scientific projects, conferences, etc. are attached. The presented materials are well designed and clearly present the candidate's past scientific activity and achievements.

2. Brief biographical details of the applicant

Dr. Tsvetelina Doncheva completed a master's program in Organic and Chemical Technologies (biotechnologies, synthesis, isolation and structural characterization of organic compounds) in 1998 at the University of Chemical Technology and Metallurgy (HTMU), Sofia. In 1999, she was appointed as a chemist in the "Chemistry of Natural Products" laboratory of IOCCP-BAS and in 2005 she was enrolled in an open doctoral program in the same laboratory. She successfully defended her dissertation work in 2010 to obtain the educational and scientific degree "doctor" at IOCCP-BAS. From 2010 until now, she has held the academic position of Assistant Professor in the "Chemistry of Natural Products" laboratory of IOCCP-BAS.

Dr. Tsvetelina Doncheva is a participant in 14 research international and national projects, and she is the leader of 3 of them. The results of her scientific research are the subject of 29 publications, of which 22 are in refereed scientific journals, a chapter of a book and a utility model, and the noticed citations are 200. Dr. Tsvetelina Doncheva is the supervisor of two graduate students and a consultant of one graduate PhD student from abroad (Mongolia). She is a member of the Bulgarian Phytochemical Society and a reviewer of numerous articles in scientific journals.

3. Fulfillment of the requirements for holding the academic position "Associate Professor"

To participate in the current competition, Dr. Tsvetelena Doncheva has submitted a certificate of fulfillment of the minimum requirements for holding the academic position "Associate Professor" at IOCCP-BAS, as follows:

Group A - 50 points (required **50 points**): PhD thesis „Alkaloid composition of species of tribe Datureae“ (2010) to obtain the PhD degree in a professional field: 4.2. Chemical sciences, scientific specialty: 01.05.10. "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" at IOCCP - BAS.

According to indicator 4 in group B - 100 points (required **100 points**): A total of 6 publications (2-Q2 and 4-Q3) in international journals are presented (*Biochemical Systematics and Ecology*, *Natural Product Communications*, *Journal of Asian Natural Products Research* и *Compt. Rend. Acad. Bulg. Sci.*), published between 2014 and 2019, in which Dr Doncheva is the first or corresponding author.

According to indicators 7 and 8 in group G – 234 points (required **220 points**): Twelve publications (2-Q1, 5-Q2, 3-Q3 and 2-Q4) are presented in journals referenced and indexed in world-renowned databases (*PLoS ONE*, *Diversity*, *Natural Product Communications*, *Natural Product Research*, *Proc. Univers. Appl. Chem. Biotech.*, *North-Western Journal of Zoology*, *In Vitro Cell. Dev. Biol.*, *Arch. Micr. and Compt. Rend. Acad. Bulg. Sci.*) and a book chapter, most of which were published in the last three years. Dr. Doncheva is the first or corresponding author in 6 of them.

According to indicator 12 in group D – 300 points (required **70 points**): The number of citations of Dr. Doncheva's scientific publications (150) included in the competition for "Associate professor", which are available in the scientific information database Scopus is impressive.

In accordance with the requirements of the Regulations for the Development of the Academic Staff of the IOCCP for occupying the academic position "Assoc. Prof.", the candidate's h-index is 7 (required ≥ 5) according to the information in the scientific database Scopus, after excluding self-citations.

The total number of points from all indicators is **684**, with which Dr. Ts. Doncheva significantly exceeds the minimum requirements for occupying the academic position of "Associate Professor" (total number of points **440**) according to the Rules of the IOCCP-BAS.

4. Evaluation of the candidate's scientific and scientific-applied activity

Alkaloids are an important class of N-containing natural compounds characteristic of plants of the families Papaveraceae, Berberidaceae, Fumariaceae, Rutaceae, Ranunculaceae, etc. with a wide range of pharmacological actions such as antimalarial (quinine), antiasthmatic (ephedrine), anticancer (homoharringtonine), vasodilator (vincamine), analgesic (morphine), antihyperglycemic (berberine), etc. Many of them have found an application in traditional or modern medicine or are a starting point for drug discovery. Some alkaloids have psychotropic (psilocin) and stimulant (cocaine, caffeine, nicotine, theobromine) actions and can also be toxic. This class of important secondary metabolites is related to the research work of Assist. Prof. Dr. Tsvetelina Doncheva. This interdisciplinary scientific field is actual, modern and socially

relevant, given the growing interest in natural products and their use as nutritional supplements and medicinal agents in recent years.

The main direction in the scientific research of Assist. Prof. Dr. Tsvetelina Doncheva is the study of the composition, structure and biological activity of secondary metabolites isolated from unexplored, rare and/or endemic plant species and organisms. The more significant scientific contributions in this direction are:

- The alkaloid composition of *Leptopyrum fumarioides* [B2 and G8] was investigated for the first time, and 4 alkaloids of the isoquinoline type were isolated and characterized, and two of them (leptopyrine and leptofumarin) are newly discovered natural compounds. The new compounds are dimeric alkaloids containing a benzyltetrahydroisoquinoline moiety linked to 3,4-dihydroisoquinoline (leptopyrine) and aporphine and benzyloisoquinoline monomer units linked by two “head-head” and “tail-to-tail” ether bridges (leptofumarin). Potential anti-inflammatory and immunomodulatory activities were established for the new compounds [B2].

- From the crude alkaloid mixture obtained from the Balkan endemic *Hypecoum ponticum*, 6 alkaloids of the isoquinoline type (tertiary and quaternary) were isolated and identified, two of which were established for the first time in the species, and hypepontine is a newly discovered natural compound. When studying the antibacterial and antifungal activity, it was found that the quaternary alkaloid mixture exhibited better activity than the tertiary alkaloid mixture. All individual compounds possess weaker antibacterial activity compared to that in a mixture, which is probably due to synergistic interactions between the identified alkaloids [G5].

- Fourteen tetrahydroisoquinoline, benzyloisoquinoline, protopine, isopavine, benzophenanthridine and morphinine alkaloids [B3], characteristic of species of the genus *Papaver*, were isolated from the protected and local endemic species *Papaver degenii* (Pirinsky poppy). This fact, as well as the fact that *P. degenii* is a glacial relict species whose speciation took place under extremely harsh environmental conditions, indicates its relative genetic stability.

- From *Pandanus amaryllifolius* Roxb. and *Pandanus tectorius* Parkinson of Vietnamese origin were isolated and identified 9 pandan-type alkaloids, three of which were discovered for the first time in *P. tectorius* [G10]. When studying the anti-inflammatory activity in *in vitro* conditions, similarities and differences in the activity of the crude alkaloid mixtures and alkaloid fractions of both species were found, due to synergistic and/or antagonistic interactions between the alkaloids [G10].

- The dimeric alkaloid talicarpine was isolated from *Thalictrum minus* ssp. *majus* (family Ranunculaceae) of Bulgarian origin [G6]. It was found that the alkaloid and its combination with TiO₂ nanoparticles exhibited pronounced antioxidant activity at pH 8.5, which was lost and transformed into a well-expressed pro-oxidant effect at pH 7.4. Moreover, as a result of synergistic connections between the nanoparticles and talicarpine, their antibacterial effect is increased. This contribution also has an applied contribution considering the use of nanoparticles as drug carriers in the diagnosis and treatment of various diseases [G6].

- Data on the structure and biological activity of alkaloids from various plant sources and the possibilities for their future application are summarized [D2, D12 and D13].

Two of the presented works of Assist. Prof. Tsvetelina Doncheva are associated with objects that do not produce alkaloids, and with the help of GC-MS the composition of two samples of *Gentiana cruciata* [G9] and skin secretions from newt (*Tr. ivanbureschi*) [G11] was determined. Nine pentacyclic triterpenoids of ursane and oleanane type were identified in *G. cruciata*, of which 3-formyl oleanolic acid was identified for the first time from a natural source, and 3-formyl ursolic acid and ursolic acid methyl ester were new to the genus *Gentiana* [G9]. Skin secretions from *Tr. ivanbureschi* contain mainly sterol compounds, representing biosynthetic precursors of alkaloids [G11].

Another important direction in the scientific research of Assist. Prof. Dr. Tsvetelina Doncheva is the comparative analysis of the alkaloid composition of plants producing alkaloids and derivation of taxonomic and chemotypic dependencies. Studies on the alkaloid composition of plants of the genera *Glaucium*, *Fumaria*, *Coridalis*, *Alkanna* and *Hypecoum* have identified 246 alkaloids, of which 86 were discovered for the first time in the respective species. The more significant scientific contributions in this direction are:

- It has been established that there are three chemotypes of *Glaucium flavum* in Bulgaria, containing different main alkaloids. A similarity in the alkaloid profile of two populations with different geographical location profile was observed and it was suggested that the formation of a chemotype depends not only on the geographical location and environmental factors, but also on the genetic characteristics of the species [B6].

- The comparative analysis of the alkaloid profiles of *Glaucium corniculatum* of Bulgarian and Algerian origin shows that plants of Bulgarian origin produce benzophenanthridine alkaloids, like plants distributed in other regions with a temperate continental climate and are differing from the samples from Algeria (tropical climate) in which this alkaloid type was not established. It has been suggested that the biosynthesis of benzophenanthridine alkaloids in *G. corniculatum* depends on the geographical region the plant is growing in and could be used as chemotaxonomic markers to help identify the source of the plants [B4].

- Based on the obtained results of GC-MS analysis of the alkaloid composition of four species of the genus *Fumaria* (*F. kralikii*, *F. rostellata*, *F. schleicherii* and *F. thuretii*), as well as of *F. kralikii* growing under different conditions (habitats, altitude), the formation of two new chemotypic groups in the genus *Fumaria* was proposed - a group producing the largest amount of spirobenzylisoquinolines (>50% of the alkaloid mixtures) (*F. kralikii*, *F. rostellata* and *F. thuretii*) and a group containing the most - a large amount of protopine alkaloids (>40% of alkaloid mixtures) and a relatively high percentage of phthalideisoquinoline alkaloids (11-19%) (*F. rostellata* and *F. schleicherii*) [B1].

- Two species of the genus *Corydalis* were studied - *C. solida* and the Bulgarian endemic *C. slivenesis*, which has an unclear taxonomic status and is considered by some authors to be a variety or subspecies of *C. solida*. A total of 21 isoquinoline-type alkaloids were identified and *C. slivenesis* was found to have a more complex alkaloid profile. The observed differences in the alkaloid composition are important from a chemotaxonomic point of view and would contribute to clarifying the taxonomic status of the species in the genus [B5].

- The alkaloidal composition of the Balkan endemic species *Alkanna primuliflora*, *A. stribrnyi* and *A. graeca* of Bulgarian origin, which have a very limited distribution, was studied. GC-MS analysis of the crude alkaloid mixtures led to the identification of 8 pyrrolizidine alkaloids, with triangularine being the main alkaloid in all studied plants, and differences were found regarding some minor components. The observed minimal differences in the alkaloid profiles of plants from different natural populations and in different developmental phases proved that the environment and ontogeny do not significantly influence the alkaloid biosynthesis. The obtained results also have an applied contribution for the knowledge of endemic species with limited distribution in our country and would facilitate the possibilities for their conservation [G1].

- The comparative study of the alkaloid composition of some *Hypecoum* species (*H. procumbens* L., *H. ponticum* Velen and *H. imberbe* Sm.) led to identification of 10 isoquinoline alkaloids. It was found that *H. ponticum*, which some authors consider to be a synonym of *H. procumbens*, is well separated from the other species (*H. procumbens* and *H. imberbe*) due to the presence of many quaternary isoquinoline alkaloids [G4].

Two of the presented works [G3 and G7] of Assist. Prof. Tsvetelina Doncheva related comparative analysis of the alkaloid composition of the *in vitro* cultivated and *ex vitro* adapted endemic *Papaver degenii*. A significant increase in alkaloid content (5 to 6 times) was found in the *in vitro* cultivated and *ex vitro* adapted plants (aerial parts and roots) compared to the wild ones. This difference may be related to the fact that growing plants under laboratory conditions can induce stress and lead to increased levels of alkaloids produced. Four isoquinoline alkaloids were isolated from the regenerated plants, with amurensine being the main alkaloid in all analyzed reaching 63.4% of the raw alkaloid mixture in the *in vitro* cultures and 88.1% in the aerial parts of the *ex vitro* adapted plants. It was found that the addition of elicitors to the nutrient medium had a positive effect on the alkaloid content but did not cause significant changes in composition. The obtained results have also an applied contribution, as they can allow the successful application of biotechnological methods for the protection of rare and endangered species, while the *ex vitro* adapted plants could be used as a source of the alkaloid amurensine with application in the treatment of neurological diseases, such as Parkinson's and Alzheimer's diseases.

5. Evaluation of the personal contribution of the candidate

The candidate's contribution to the presented research is indisputable, since in 12 of the presented scientific works Assist. Prof. Dr. Tsvetelina Doncheva is the first and/or corresponding author. The candidate in the competition has undoubtedly developed the necessary scientific competence and skills to isolate and identify alkaloids of different skeletal types by using various chromatographic (column, thin-layer and GC-MS) and spectral (NMR and MS) methods, analyzing and summarizing the obtained results. This acquired competence has also specified the future directions for the candidate's development, formulated in the Statement of Scientific Contributions as a continuation and expansion of the previous research, namely the continuation of research on medicinal, endemic and unexplored plants, aiming at the discovery of new biologically active compounds (alkaloids) and the search for a possible structure-biological

activity relationship, research related to tracking the influence of various environmental factors on the alkaloid content in plants and developing methods for obtaining biologically active substances from medicinal plants by using modern extraction techniques.

6. Personal impressions

I personally know Assist. Prof. Dr. Tsvetelina Doncheva and I have formed excellent impressions of her professional and collegial qualities. She is a thorough and self-critical researcher with a sense of current issues in the field of phytochemistry and a worthy successor to the traditions of IOCCP in the field of alkaloid chemistry.

CONCLUSION

The documents and materials presented by Assist. Prof. Dr. Tsvetelina Doncheva meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB, the Regulations for the Implementation of the LDASRB of the Bulgarian Academy of Sciences and the Regulations of the IOCCP-BAS. The candidate in the competition has submitted a significant number of scientific works published after the materials used in the defense of the PhD thesis. The candidate's works contain original scientific and applied contributions that have received international recognition, a representative part of which has been published in journals and scientific collections issued by international academic publishing houses. The scientific qualification of Assist. Prof. Dr. Tsvetelina Doncheva is unquestionable.

The achievements of Assist. Prof. Dr. Tsvetelina Doncheva in the research activity fully correspond to the specific requirements of the Regulations of IOCCP-BAS for application of LDASRB.

After getting acquainted with the materials and scientific works presented in the competition, analyzing their significance and their scientific, scientific-applied and applied contributions, I find it reasonable to give my positive assessment and recommend the Scientific Jury to prepare a report-proposal to the Scientific council of IOCCP-BAS for the election of Assist. Prof. Dr. Tsvetelina Doncheva in the academic position of "Associate Professor" at IOCCP-BAS in professional direction 4.2. Chemical sciences, scientific specialty "Bioorganic chemistry, chemistry of natural and physiologically active substances".

26.03.2024

Reviewer:

(Prof. Dr Antoaneta Trendafilova)