ANNOTATION OF THE MATERIALS

under art. 76 of the Regulations for the Development of academic staff of PU "Paisii Hilendarski", including

EXTENDED HABILITATION REFERENCE

Assoc. Prof. Plamen Stefanov Stoyanov, PhD

for participation in a competition for the academic position "Professor", announced in State Gazette, issue 92/18.11.2022

Field of higher education 4. Natural Sciences, Mathematics and Informatics,professional field 4.3 Biological sciences (Pharmaceutical Botany)

The presented materials for participation in the competition for the academic position "Professor" include 21 scientific publications, 2 book chapter, 3 textbooks and 2 study guide (which are not used for the acquisition of PhD and the academic positions "Assistant" and "Associate Professor")

- All presented publications are referenced and indexed in the world-renowned scientific information databases Web of Science and Scopus
- Total number of IF for the articles participating in the competition: 25.43
- Total number of citations for the competition (only in Web of Science и Scopus): 101
- h-index according to Scopus: 5 https://www.scopus.com/authid/detail.uri?authorId=55820445700
- h-index according to Web of Science: 5 https://www.webofscience.com/wos/woscc/citation-report/8f732da4-6dc6-4ff7-b1ff-2090d7bc8f9c-69bc257f
- ORCID №: https://orcid.org/0000-0002-5631-1444
- Google scholar: https://scholar.google.bg/citations?user=msRneoMAAAAJ&hl=bg&authuser=1
- Researchgate: https://www.researchgate.net/profile/Plamen-Stoyanov

For Criterion A.

SCIENTIFIC PUBLICATIONS ON THE BASIS OF WHICH THE DISSERTATION WORK IS DEFENDED:

1. **Stoyanov, P.,** Belkinova, D., Mladenov, R., Teneva, I. Analysis of the water in the reservoirs Krushovitsa, Enitsa and Valchovets (Northern Bulgaria) for presence of cyanotoxins. In: PU “Paisii Hilendarski”, Jubilee Proceedings “Biological sciences for a better future”, ISSN 1312-062X, University of Plovdiv Publishing House, 2012, 237-249.

   Abstract. This study presents data on the dominant species Cyanoprokaryota and results from performed toxicological analysis of the water regarding presence of cyanotoxins in three relatively small reservoirs located in Pleven district (Northern Bulgaria): Krushovitsa reservoir, Valchovets reservoir and Enitsa reservoir. Cyanoprokaryotic species *Planktothrix agardhii, Anabaena spiroides* and *Aphanizomenon fl os-aquae*, reported repeatedly as producers of hepato- and neurotoxins, were dominant in the studied reservoirs with bloom concentrations from 1,03 mg/L to 10,5 mg/L. Analysis of

the water samples for presence of cyanotoxins was conducted using ELISA kits for microcystins/nodularins and for saxitoxins, HPLC, as well as *in vitro* cytotoxicity tests. Microcystins/nodularins were detected in the water samples collected from Enitsa reservoir and saxitoxins were found in the water samples from Valchovets reservoir.

2. **Stoyanov, P.,** Teneva, I., Mladenov, R., Belkinova, D. (2013). Diversity and ecology of the phytoplankton of filamentous blue-green algae (Cyanoprokaryota, Nostocales) in Bulgarian standing waters; *Ecologia Balkanica*, 5(2): 1-6.

   Abstract: The current study presents data about the diversity and ecology of filamentous blue-green algae, found in the phytoplankton of 42 standing water basins in Bulgaria. We identified 9 species from Cyanoprokaryota, which belong to 5 genera from order Nostocales. Ecological characterization of the identified species has been performed. Data about the physicochemical parameters of the water basins are also provided.

3. **Stoyanov, P.,** Moten, D., Mladenov, R., Dzhambazov, B., Teneva, I. (2014). Phylogenetic relationship of some filamentous cyanoprokaryotic species. *Evolutionary Bioinformatics*, (10): 39-49. Web of Science $Q3_{IF\ 1.452}$, Scopus $Q2_{\ SJR\ 0.703}$

   Abstract: The polyphasic approach is the most progressive system that has been suggested for distinguishing and phylogenetically classifying Cyanoprokaryota (Cyanobacteria/Cyanophyta). Several oscillatorialean genera (*Lyngbya*, *Phormidium*, *Plectonema*, and *Leptolyngbya*) have problematic phylogenetic position and taxonomic state because of their heterogeneity and polyphyletic nature. To accurately resolve the phylogenetic relationship of some filamentous species (*Nodosilinea bijugata*, *Phormidium molle*, *Phormidium papyraceum*), we have performed phylogenetic analyses based on 16S rRNA gene and the phycocyanin operon (PC-IGS) by using maximum-likelihood (ML) tree inference methods. These analyses were combined with morphological re-evaluation. Our phylogenetic analyses support the taxonomic separation of genus *Nodosilinea* from the polyphyletic genus *Leptolyngbya*. Investigated *Nodosilinea* strains always formed a coherent genetic cluster supported with a high bootstrap value. The molecular phylogeny confirmed also the monophyly of the *Wilmottia* group. In addition, data reveal that although *P. papyraceum* is morphologically similar to *Wilmottia murrayi*, this species is genetically dis-tinct. Strains from the newly formed genus *Phormidesmis* and some *Phormidium priestleyi* strains were clustered in a separate clade different from the typical *Phormidium* species, but without strong bootstrap support.

4. Teneva, I., Gecheva, G., Cheshmedjiev, S., **Stoyanov, P.**, Mladenov, R., Belkinova, D. (2014). Ecological status assessment of Skalenski Lakes (Bulgaria). *Biotechnology & Biotechnological Equipment*, 28(1): 82-95. Web of Science $Q4_{IF\ 0.300}$, Scopus $Q4_{\ SJR\ 0.173}$

   Abstract: Over the past decade new ecological indices based on phytoplankton and macrophytes were developed as part of the tools for assessment of the ecological status of water bodies. This study demonstrates the applicability of two of them (Assemblage index /Q/ and Algae Group Index /AGI/) for evaluation of water bodies from a lake type L4 as well as their comparability. Assessment of the ecological status of two lake ecosystems was performed in order to ensure successful protection, enhancement and management of lowland and semi-mountain lakes in Bulgaria. Data on the aquatic flora from Golyamo Skalensko Lake and Malko Skalensko Lake over a period of two years were used to assess their ecological status. In addition, the toxic potential of the established dominant cyanoprokaryotic species was also evaluated. Phytoplankton- and macrophytebased metrics resulted in complementary evaluation of temporary and long-term environmental conditions. Despite the hydraulic connection and proximity between the two lakes, Golyamo Skalensko Lake and Malko Skalensko Lake appear as completely different ecosystems, according to the phytoplankton structure (species composition, number of species, abundance and seasonal succession), macrophytes and ecological status.

For Criterion B4.

HABILITATION WORK - SCIENTIFIC PUBLICATIONS IN PUBLICATIONS THAT ARE REFERENCED AND INDEXED IN SCIENTIFIC INFORMATION DATABASES OF WEB OF SCIENCE AND SCOPUS:

1. Dimitrova-Dyulgerova, I., Marinov, Y., Mladenova, Ts., **Stoyanov, P.,** Stoyanova, A. (2019). Essential Oils Composition of the Endemic Bulgarian Plant Species *Micromeria frivaldszkyana* (Degen) Velen. (Lamiaceae). *Comptes rendus de l'Académie bulgare des Sciences*. 72(11): 1484-1491. Web of Science Q4 $_{IF\ 0.343}$, Scopus Q2 $_{SJR\ 0.218}$

   Abstract. Essential oils from the leaves and flowers of *Micromeria frivaldszkyana* obtained by hydrodistillation were analyzed by GC/MS for the first time. The yield of oils during the flowering stage was 0.69% in flowers and 0.26% in leaves. Thirty components were identified in the flower oil, representing 98.45% of the total content, with the main constituents over 3% pulegone (45.05%), germacrene D (18.43%), limonene (9.68%), γ-elemene (8.77%) and p-menthone (6.33%). Twenty-four were the components in leaf oil, representing 98.45% of the total content, with constituents over 3% pulegone (42.61%), p-menthone (28.33%), germacrene D (8.31%); limonene (5.99%), γ-elemene (4.56%) and menthol (3.51%). Oxygenated monoterpenes were the most represented group in these oils, followed by sesquiterpene hydrocarbons and monoterpene hydrocarbons. In the autumn, leaf oil (yield 0.24%) sesquiterpene hydrocarbons were predominant aroma substances (the main β-caryophyllene – 52.92%). In October 16 different components in leaf oil were identified, among which over 3% were: α-humulene (7.07%) and caryophyllene oxide (5.30%).

2. Dinev, T., Rusenova, N., Tzanova, M., Grozeva, N., Gerdzhikova, M., **Stoyanov, P.,** Mladenova, Ts., Beev, G. (2020). Antimicrobial Potential of Methanolic Extracts from *Betonica bulgarica* Degen et Neič. (Lamiaceae). *Ecologia Balkanica*, 12(2): 165-174. Web of Science, Scopus Q4 $_{SJR\ 0.144}$

   Abstract. *Betonica bulgarica* Degen et Neič. (syn. *Stachys bulgarica* Hayek) is a Bulgarian endemic plant included in Red Data Book of Bulgaria under the category "endangered". The aim of the present study is to provide data about the antimicrobial activity of *B. bulgarica* leaf, flower, seed, stem and root methanolic extracts against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus cereus, Aspergillus ochraceus* 2002 IM-BAS, *Fusarium moniliforme* 394 FN-9, *Fusarium graminearum* 2294 IMI 155426 and *Penicillium verrucosum* 2003 NRRL F-143. Antimicrobial activity of the extracts was evaluated by agar well diffusion method. Root extracts of *B. bulgarica* exhibited the highest antibacterial activity against *S. aureus* and *B. cereus* with large zones of inhibition. All extracts demonstrated either low and statistically insignificant activity against *E. coli* or a lack thereof. As a whole, extracts of Ablanovo area (in Sinite kamani National Park) exerted the highest activity against *S. aureus, B. cereus* and *E. coli*. Leaf, flower, stem and root extracts of *B. bulgarica* showed either a lack of antifungal activity or low and statistically insignificant one.

3. Mladenova, Ts., **Stoyanov, P.,** Todorov, K., Davcheva, D., Kirova, G., Deneva, T., Gyuzeleva, D., Mladenov, R., Bivolarska, A. (2021). Phytochemical and Biological Traits of Endemic *Betonica bulgarica* (Lamiaceae). *Separations*, 8(2): 11. Web of Science Q2 $_{IF\ 3.344}$, Scopus Q3 $_{SJR\ 0.418}$

   Abstract: *Betonica bulgarica* is an endemic species distributed in Bulgaria. The chemical composition of the essential oil analysed by GC–MS (Gas chromatography–mass spectrometry) and the content of trace elements analysed by ICP–MS (Inductively coupled plasma mass spectrometry) were determined. Additionally, a study on the types and distribution of trichomes was done using a microscope with a camera. The essential oil was characterized using a high concentration of sesquiterpene hydrocarbons, whose major compounds are ß-caryophyllene (17.4%), germacrene D (9.9%), and β-bourbonene (6.7%). The contents of manganese (177.2 μg/g) and strontium (156.8 μg/g) were highest among the investigated micronutrients. Two types of trichomes were identified on the adaxial and abaxial

epidermises of the leaves of *B. bulgarica* - covering and glandular. Peltate stacked glandular trichomes with a four-celled head of type B were observed on the leaf surface.

4. Mladenova, Ts., **Stoyanov, P.,** Denev, P., Dimitrova, S., Katsarova, M., Teneva, D., Todorov, K., Bivolarska, A. (2021). Phytochemical composition, antioxidant and antimicrobial activity of the Balkan endemic *Micromeria frivaldszkyana* (Degen) Velen. (Lamiaceae). *Plants*, 10(4): 710. Web of Science Q1 $_{IF\ 4.658}$, Scopus Q1 $_{SJR\ 0.765}$

   Abstract: The current study investigates the content of sugars, organic acids, phenolic acids and flavonoids, as well as antioxidant and antimicrobial activity of Balkan-endemic Micromeria frivaldszkyana. Glucose was the most abundant sugar in the plant (2.77%), followed by fructose (1.18%) and galactose (0.82%). Eight organic acids were detected with quinic acid being in the highest content—556.3 mg/100 g DW. From the individual phenolic acids, rosmarinic acid was found in the most significant amounts (2040.1 ± 1.97 mg/100 g) and hesperidin was the major representative of flavonoids with content 131.2 ± 5.6 mg/100 g DW. The antioxidant activity of the plant was studied by six methods: 2,2´-diphenylpicrylhydrazyl (DPPH)—286.4 ± 10.43 mM TE/g, 2,2´azinobis (3)-ethylbenzthiazoline-6-sulfonic acid (ABTS)—358.4 ± 10.4 mM TE/g, ferric reducing antioxidant power (FRAP)—388.0 ± 32.4 mM TE/g, cupric reducing antioxidant capacity (CUPRAC)— 905.6 ± 19.2 mM TE/g, Oxygen Radical Absorbance Capacity (ORAC)—3250.5 ± 208.1 µmol TE/g and Hydroxyl Radical Averting Capacity (HORAC)—306.1 ± 23.5 µmol GAE/g. In vitro antimicrobial activity against nine microorganism was evaluated but the extract displayed antimicrobial activity only against Listeria monocytogenes ATCC 19111 with inhibition zone diameter 9 mm and minimal inhibitory concentration (MIC) 10 mg/mL.

5. Mladenova, T., Batsalova, T., Dzhambazov, B., Mladenov, R., Teneva, I., **Stoyanov, P.,** Bivolarska, A. (2022). Antitumor and Immunomodulatory Properties of the Bulgarian Endemic Plant *Betonica bulgarica* Degen et Neič. (Lamiaceae). *Plants*, 11, 1689. Web of Science Q1 $_{IF\ 4.658}$, Scopus Q1 $_{SJR\ 0.765}$

   Abstract: Background: Extracts obtained from different Betonica species have been shown to possess important biological properties. The present study aimed to investigate the cytotoxicity, antitumor and immunomodulatory potential of the endemic plant Betonica bulgarica (Lamiaceae) and thus, reveal new aspects of its biological activity. **Methods:** Methanolic extract obtained from inflorescences was analyzed for cytotoxicity against mammalian cell lines. The antitumor potential of the sample was determined using human cervical and lung adenocarcinoma cells (HeLa and A549). Programmed cell death-inducing effects against HeLa cells and peripheral blood lymphocytes, as well as immunomodulatory properties of the extract were determined by flow cytometry analysis. **Results:** The research results demonstrated that the extract has significant inhibitory potential against HeLa cells (mean IC50 value 119.2 µg/mL). The sample selectively induced apoptotic death in tumor cells. Cytotoxic effects towards mouse cell lines were detected following treatment with high concentrations of Betonica bulgarica extract (200 and 250 µg/mL). Twenty-four-hour ex vivo incubation of peripheral blood leucocytes in growth medium containing plant extract induced prominent effects in distinct immune cell populations. They included elevated levels of $CD25^+$ and $CD56^+$ T cells' lymphocytes, particularly $CD4^+CD25^+$ and $CD8^+CD56^+$ cells. **Conclusions:** The present study demonstrates that Betonica bulgarica inflorescence extract possesses potential beneficial antitumor and immunomodulatory activity and could serve as a source of bioactive compounds with biomedical application.

For Criterion G7.

SCIENTIFIC PUBLICATIONS IN JOURNALS THAT ARE REFERENCED AND INDEXED IN THE SCIENTIFIC INFORMATION DATABASES OF WEB OF SCIENCE AND SCOPUS, OUTSIDE OF THE HABILITATION THESIS

1. **Stoyanov**, **P.,** Dimitrova-Dyulgerova, I., Teneva, I., Todorov, K., Mladenov, R. (2015). Inventory of Pteridophytes on the Territory of "Bulgarka" Nature Park. *Ecologia Balkanica*, 7(2): 29-34. Web of Science, Scopus

   Abstract. This study reports data on the diversity of Pteridophyte of the "Bulgarka" Nature Park. Twenty-nine species belonging to the divisions Lycopodiophyta, Equisetophyta and Polypodiophyta were identified, including six new species to the park: *Asplenium onopteris, Dryopteris dilatata*, *Equisetum palustre*, *Huperzia selago*, *Ophioglossum vulgatum* and *Polystichum lonchitis.* Among the identified species the ferns were prevailing. Fifteen species were medicinal plants and eight species have conservation significance. The status of the populations and major threats to the habitats were discussed.

2. **Stoyanov, P.,** Raycheva Tz., Karaycheva G., Mladenova, Ts. (2019). Current state of collections of genus *Asparagus* in Bulgarian national's herbaria. *Ecologia Balkanica*, 11(1): 27-33**.** Web of Science, Scopus Q4 SJR 0.134

   Abstract. Base on the national herbariums deposited in the country and some authors' collections, a review of the status of *Asparagus* species has been made. Information on ecology, height range and representation of Bulgarian species in herbarium collections is updated. The information is organized in a relational database, which is an important information resource and a starting point for future taxonomic and chorological studies. This study reveals the state of the art and summarizes the gaps and problems.

3. Mladenova, Ts., **Stoyanov, P.,** Michova-Nankova, I. Mladenov, R., Boyadzhiev, D., Bivolarska, A., Todorov, K. (2019). Comparative Leaf Epidermis Analyses of *Micromeria frivaldszkyana* (Degen) Velen. and *Clinopodium vulgare* L. (Lamiaceae) from Bulgarka Nature Park, Bulgaria. *Ecologia Balkanica*, 11(2): 133-140. Web of Science, Scopus Q4 SJR 0.134

   Abstract. The present work presents a comparative anatomical study of the leaf epidermis of the Bulgarian endemic *Micromeria frivaldszkyana* (Degen) Velen. and *Clinopodium vulgare* L., belonging to the family Lamiaceae. In both species they occur diacytic and anomocytic stomatal type, while the indumentum is presented by multicellular, linear covering trichomes and multicellular, stacked glandular trichomes. The results obtained from the statistical data processing contribute to the distinction between the two species with respect to the peculiarities of the leaf epidermis. Taxonomically significant anatomical features for their determination are: number, width and length of stomata on the abaxial epidermis; width of on the adaxial epidermis; number, width and length of basic epidermal cells along both epidermis and thickness of upper and lower cuticle.

4. **Stoyanov, P.,** Dimitrova-Dyulgerova, I., Radoukova, Tz., Mladenov, R. (2019). Floristic Diversity of Certain Wetlands in Southern Bulgaria. *Ecologia Balkanica*, 11(2): 141-153. Web of Science, Scopus Q4 SJR 0.134

   Abstract. The two-year study on the species composition of higher plants was conducted in three moisture zones in the Maritsa river valley, Bulgaria: "Rice-field Plovdiv", protected zone (PZ) "Rice-field Tsalapitsa" and protected area (PA) "Martvitsata Zlato Pole". The analysis was done, using the floristic methods. There were 154 species of vascular plants identified, which belong to 125 genera and 43 families. The highest floristic diversity was found for PA "Martvitsata Zlato Pole" – 74% of the total number of species found, followed by "Rice-field Plovdiv" (47%) and PZ "Rice-field Tsalapitza" (36%).

The families Asteraceae, Poaceae, Fabaceae and Lamiaceae have the largest number of representatives. The total floristic composition of the three tested areas showed the predominance of dicotyledonous taxa. The comparative analysis of the biological types showed the prevalence of the perennial herbaceous plants, followed by the annual plants.

5. Gecheva, G., Belkinova, D., Hristeva, Y., Mladenov, R., **Stoyanov, P.** (2019). Phytoplankton and macrophytes in Bulgarian standing water bodies. *Ecologia Balkanica*, Special Edition 2: 45-61. Web of Science, Scopus Q4 $_{SJR\ 0.134}$

Abstract. The current status of a lake can be evaluated via monitoring based on biological quality elements. Reference aquatic flora communities reflect pristine situations that exist or would exist with no or very minor disturbances from anthropogenic pressure. Phytoplankton and macrophytes were studied in 10 national lake types (L1, L3, L4, L5, L7, L8, L11, L12, L13, L17). Type-specific taxa and groups character in reference and near reference conditions were described. Abiotic parameters (water chemistry) were also discussed. Descriptor species from 25 FGs were registered in phytoplankton communities in lakes in reference and near reference conditions. Dinoflagellates (L0) cryptomonads (Y) and various benthic/periphytic taxa (MP) were distributed in almost all lake types. Character descriptor species and FGs were reported for lake types L1 and L5. Motile mixotrophic dinoflagellates (L0) and cryptomonads (Y) had highest relative biovolume in ultraoligotrophic alpine lakes (L1) due to their ability to utilize effectively scarce trophic resources. Motile euglenoids (W1, W2), small-celled colonial Cyanobacteria (K), green algae and small cryptomonads (X1, X2), coccal green algae (J) and meroplanctonic diatoms (MP) dominated phytoplankton community in riverine marshes (L5). Recorded FGs from natural lakes were also common in and their analog among heavy modified water bodies: shallow lowland reservoirs L17. Phytoplankton communities of L13 (small and medium-size semi-mountain reservoirs in the Eastern Balkans) were more similar with those of L11 (large deep reservoirs) and mountain L1, L3. Further surveys are needed in order to classify specific features of phytoplankton communities in L3, L4, L7, L11 and L12.

6. Teneva, I., Belkinova, D., Mladenov, R., **Stoyanov, P.,** Moten, D., Basheva, D., Kazakov, S., Dzhambazov, B. (2020). Phytoplankton composition with an emphasis of Cyanobacteria and their toxins as an indicator for the ecological status of Lake Vaya (Bulgaria) – part of the Via Pontica migration route. *Biodiversity Data Journal* 8: e57507. Web of Science Q3 $_{IF\ 1.225}$, Scopus Q2 $_{SJR\ 0.509}$

Abstract. As producers of biomass, cyanobacteria are a major part of the phytoplankton in a large number of water basins. Due to the cyanobacterial blooms and cyanotoxins produced, these organisms are recognized as a threat and ecological risk for water bodies. Released cyanotoxins may cause death of many organisms including birds and fish. Vaya Lake is the largest natural lake in Bulgaria. It is located on the Via Pontica migration route of birds between Europe and Africa. Since 2003, the lake has been declared as a "Wetland of international importance" under the Ramsar Convention. According to the literature data from 2002-2006, the Lake is defined as highly eutrophied due to strong anthropogenic pressure, but regular monitoring of the cyanobacterial blooms and presence of cyanotoxins after this period is missing. Taking into account the importance of this unique, protected ecosystem, our aim was to make a complete ecological assessment of the present state of Lake Vaya by using the phytoplankton, with an emphasis on cyanobacterial blooms and the presence of cyanotoxins. As results of the study, we 1) characterized the phytoplankton composition qualitatively and quantitatively; 2) evaluated the ecological status of the western and eastern part of the Lake; 3) identified the potential producers of cyanotoxins; 4) observed cyanobacterial blooms and discussed the influence of macrophytes on their spread; 5) measured the concentrations of the cyanotoxins MCs, CYL and STXs in water samples from both parts of the Lake. Our results indicated the need for continued observation of cyanobacterial composition, blooming and the presence of cyanotoxins in Lake Vaya.

7. Tomova, T., Petkov, V., Slavova, I., **Stoyanov, P.,** Argirova, M. (2020). Naturally present metal ions in plants could interfere with common antioxidant assays. *MethodsX*, 7, 1-7. Web of Science Q2 JCI 0.43, Scopus Q2 SJR 0.356

   Abstract. Most of the commonly applied assays used to assess antioxidant properties of plant extracts exploit the ability of some biologically active metabolites to participate in oxidation-reduction reactions with metal ions. On the other hand, most plants contain different chelated metal ions whose levels depend on the geographic origin, soil, and environmental pollutions. In this study the levels of redox-active metal ions in three plant sources were measured and extracts of these botanicals were treated with Chelex^{R} –an ion exchanger that is noteworthy for its ability to bind transition metal ions. The original and chelated extracts were subjected to three antioxidant assays based on single electron transfer. The results obtained showed statistically significant differences between the original and Chelex-treated extracts suggesting that the naturally present metal ions could interfere with the results of the three most commonly applied antioxidant methods.

8. Popova, M., Esad, M., Mladenova, Ts., **Stoyanov, P.,** Bivolarska, A. (2021). Biochemical aspects of the effect of Silymarin on lipid metabolism and glucose homeostasis. *General medicine*, 23(2): 45-50. Web of Science, Scopus Q4 SJR 0.102

   Abstract. The regulation of lipid metabolism and glucose homeostasis is conducted through numerous processes and signaling pathways. Disorders in them are the reason for the development of a number of socially significant diseases. Silymarin is extracted from the plant *Silybum marianum* (milk thistle). It has many positive effects (antioxidant, anti-inflammatory, anti-tumor, antifibrotic, immunomodulatory, etc.), which make it a potential adjunct in the treatment of these conditions. Silymarin enhances lipolysis, inhibits TAG synthesis and lipogenesis, lowers levels of “bad” cholesterol and increases those of “good” cholesterol, thus improving lipid metabolism. These effects are manifested by milk thistle, affecting a number of enzymes – phosphatidate phosphatase (PAP), fatty acid synthase (FAS), diacylglycerol acyltransferase-1 (DGAT1) and others and transcription factors such as PPARα (peroxisome proliferator-activated receptor alpha). It regulates cholesterol levels through a double mechanism – suppression of its synthesis and the effect of resin in the entero-hepatic cycle. Regarding glucose homeostasis, milk thistle normalizes blood sugar levels, lowers glycated hemoglobin (HbA1c) levels and improves glucose tolerance. It manifests its effects through various mechanisms – supports the regeneration of β-cells of the pancreas, regulates the signaling pathway of phosphatidylinositol-3-kinase (PI3K), inhibits major enzymes of gluconeogenesis. The numerous beneficial properties of silymarin make it the focus of alternative medicine and a means of adjunctive therapy to statin therapy. However, more studies and clinical trials are needed to clarify its full potential and mechanism of action.

9. Georgieva, Y., Katsarova, M., **Stoyanov, P.,** Mladenov, R., Denev, P. Teneva, D., Plotnikov, E., Bozov, P., Dimitrova, S. (2021). Metabolite Profile and Antioxidant Activity of Some Species of Genus *Scutellaria* Growing in Bulgaria. *Plants*, 10(1): 45. Web of Science Q1 IF 4.658, Scopus Q1 SJR 0.765

   Abstract: Until now, the interest to plants from genus *Scutellaria* in Bulgaria has been focused mainly on the terpenes in them. The purpose of this study is to enrich the information on the composition of the Bulgarian Scutellaria species in terms of both polyphenolic content as well as primary metabolites such as mono-, oligosaccharides and organic acids. An aerial part of three *Scutellaria* species growing in four low mountain regions of Southern Bulgaria was used. The flavonoids scutellarin, baicalin, baicalein, wogonin, wogonoside, luteolin, chrysin and a caffeoyl phenylethanoid glycoside-verbascoside have been identified via HPLC in different extracts from *Scutellaria altissima*, *Scutellaria albida* and *Scutellaria galericulata*. The antioxidant activity of the extracts has been evaluated. The *Scutellaria altissima* from Mezek and *Scutellaria galericulata* from Parvenets we studied, which are the richest in flavonoids (represented mainly by baicalin, scutellarin and wogonoside), show the highest Oxygen Radical Absorption Capacity. Hydroxyl Radical Averting Capacity of *Scutellaria albida* from Mezek and *Scutellaria altissima* from Bachkovo is the most pronounced, probably due to the content of scutellarin

and luteolin and chrysin, respectively. Antioxidant activity of aqueous, methanolic and 70% and 96% ethanol extracts were also determined by the electrochemical method.

10. Raycheva, Ts., Stoyanov, K., **Stoyanov, P.** (2021). *Rumex kerneri* Borbás (Polygonaceae) in the Bulgarian flora – Morphology, Leaf Epidermis, Pollen Morphology and Karyology. *Ecologia Balkanica*, 13(1): 119-130**.** Web of Science, Scopus Q4 SJR 0.137

Abstract. The current study provides detailed information about *Rumex kerneri* Borbas in the flora of Bulgaria. The data about this species in the floristic literature is contradictory and uncompleted. The morphologically closely allied species *R. cristatus* DC. and *R. patientia* L. misleads in the determination of the species. Both species are used as a referent to establish the discrete morphological characters and metrics. The current study provides detailed information about pollen and fruit morphology, leaf epidermis, and karyology of *Rumex kerneri* Borbas in the flora of Bulgaria. The data about this species in the floristic literature is contradictory and uncompleted. The most reliable characters in the differentiation between *R. cristatus* and *R. patientia* the reduced number of flowers in a cluster of *R. kerneri*, the development of only one tubercle in the mature valves, and the abaxial surface of the leaf blade. Also, the pollen morphology (light and scanning electron microscopy) was confirmed in this study. The somatic chromosome number of the species 2*n* = 80 is also given. This is the first chromosome number for the taxon from Bulgaria and confirms the earlier reports.

11. Petkov, V., Ardasheva, R., Prissadova, N., Kristev, A., **Stoyanov, P.,** Argirova, M. (2021). Receptor-mediated biological effects of extracts obtained from three *Asplenium* species. *Zeitschrift Für Naturforschung C*, 76(9-10): 367–373. Web of Science Q4 IF 1.885, Scopus Q3 SJR 0.332

Abstract: This study reports the effects of aqueous extracts obtained from three fern species of Bulgarian origin: *Asplenium ceterach* L., *Asplenium scolopendrium* L., and *Asplenium trichomanes* L. on the contractility and bioelectrogenesis of rat gastric smooth muscle tissues. In the concentration range 0.015–0.150 mg/mL the three extracts contracted smooth muscle tissues in a concentration-dependent manner. The contractions caused by *A. ceterach* L. and *A. scolopendrium* L. extracts (0.150 mg/mL) were reduced by ketanserin (5×10^{-7} and 5×10^{-6} mol/L), an antagonist of serotonin 5-HT2 receptor. The contraction evoked *by A. trichomanes* L. (0.150 mg/mL) was significantly reduced by 1×10^{-6} mol/L atropine, an antagonist ofmuscarinic receptors, and turned into relaxation against the background of 3×10^{-7} mol/L galantamine. After combined pretreatment with galantamine and L-arginine (5×10^{-4} mol/L), this relaxation become more pronounced. The study demonstrates that constituents of *A. ceterach* L. and *A. scolopendrium* L. extracts act as agonists of 5-HT2 receptors and cause contraction by activating serotonergic signaling system. A*. trichomanes* L.-induced reaction is an additive result of two opposite-in-character effects. The dominant contraction is initiated by inhibition of acetylcholinesterase activity. The relaxation develops with pre-inhibited acetylcholinesterase, it is significantly potentiated by L-arginine, and therefore associated with nitrergic signaling pathway.

12. Petkov, V., Batsalova, T., **Stoyanov, P.,** Mladenova, T., Kolchakova, D., Argirova, M., Raycheva, T., Dzhambazov, B. (2021). Selective Anticancer Properties, Proapoptotic and Antibacterial Potential of Three *Asplenium* Species. *Plants*, 10, 1053. Web of Science Q1 IF 4.658, Scopus Q1 SJR 0.765

Abstract: The ferns *Asplenium ceterach* L., *Asplenium scolopendrium* L. and *Asplenium trichomanes* L. have wide application in traditional medicine worldwide. However, the scientific research on their anticancer and antibacterial properties is insufficient. The present article aims to provide more information on this topic. Extracts derived from the aerial parts of *A. ceterach*, *A. scolopendrium* and *A. trichomanes* were examined using a panel of in vitro assays with different bacterial and mammalian cells. The cytotoxicity and anticancer activity of the samples were analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Trypan blue assays with three human (A549, FL, HeLa) and three murine (3T3, TIB-71, LS48) cell lines. Inhibitory effects on the growth of Grampositive (*Bacillus cereus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria were determined by the agar

diffusion assay. Apoptosis-inducing properties of the extracts were analyzed by flow cytometry. Superoxide dismutase (SOD) activity in extract-treated cells was investigated by ELISA. The obtained results demonstrate selective anticancer activity of all three *Asplenium* species. The extract from *A. ceterach* displayed the strongest inhibitory properties against human cervical cancer cells and bacterial cells. It induced a lower level of cytotoxicity against mouse cell lines, indicating a speciesspecific effect. The extract from *A. trichomanes* demonstrated better anticancer and antibacterial properties than the sample from *A. scolopendrium*. Further experiments linked the mechanism of action of *A. ceterach* extract with oxidative stress-inducing potential and strong proapoptotic potential against the cervical cancer cell line HeLa. *A. trichomanes* and *A. scolopendrium* extracts appeared to be potent inducers of necrotic cell death.

13. Petkov, V., Slavova, I., Teneva, D., Mladenova, Tz., **Stoyanov, P.,** Argirova, M. (2021). Phytochemical Study and Biological Activity of Three Fern Species of the *Asplenium* Genus Growing in Bulgaria. *Natural Products Journal*, 11. Web of Science Q4 JCI 0.21, Scopus Q3 SJR 0.203

Abstract: Background: Ferns are underestimated as medicinal plants and their use in traditional medicine is limited despite their diversity. Most of the research on their healing properties and phytochemical composition related to their biological activity has been focused on the secondary metabolites synthesized by ferns. **Objective:** In this study, we aimed to make an in-depth chemical characterization of three ferns widely spread in Bulgaria – *Asplenium ceterach L*., *Asplenium scolopendrium L*. and *Asplenium trichomanes L*. **Methods:** Micro elemental analysis was carried out using ICP-MS. Standard laboratory methods were used to determine the content of proteins, fats and fatty acids. Chromatographic methods were applied to quantify some secondary metabolites. Two antioxidant methods and two antimicrobial tests were used to evaluate the biological properties of the ferns tested. **Results:** Micro elemental analysis showed that these ferns could be useful source of zinc and iron. Protein content slightly varied among the species (13.6% – 18.4% of the dried plant weight). Negligible was the variation in oil content (3.1% – 4.0%, dry weight base); oil composition was dominated by saturated fatty acids. Some principal classes secondary metabolites, individual phenolic acids and flavonoids were quantified. The extract obtained from *Asplenium ceterach L*. demonstrated the highest antioxidant capacity. Fern extracts showed moderate antimicrobial activity against six of the 14 microorganisms tested. **Conclusion:** The results obtained may reveal new areas of application for ferns and are a solid basis for comparison with the same species growing in other geographical and climatic conditions, which may affect their healing properties.

14. Raycheva, Ts., **Stoyanov, P.,** Todorov, K., Raycheva, T. (2021). Vascular flora of railway junctions in the Upper Tracian Lowland (Bulgaria). *Ecologia Balkanica*, 13(1): 45-53 Web of Science, Scopus Q4 SJR 0.137

Abstract. Floristic studies have been carried out in the Upper Thracian Lowland in 14 railway stations: Dimitrovgrad, Knizhovnik, Malevo, Maslinovo, Most, Stara Zagora, Yabalkovo, Uzundzhovo,Harmanli, Skobelevo, Karadzhalovo, Stalevo, Plovdiv and Haskovo during the period 2017 to 2019. A total of 267 species, 174 genera and 53 families of higher vascular plants were identified. Alien and invasive species make up for a relatively high percentage of 9.4% in the railway flora. This indicates that the railway network plays an important role in the penetration and further dispersal of alien species in Bulgarian. The long-distance spread of railway areas is most common with passing trains and people moving. The largest number of alien species were found at stations with more intensive movement of passengers and cargo, where maneuvers are erformed, stay of freight trains, such as Dimitrovgrad, Haskovo, Plovdiv, Stara Zagora. The alien species found at several of the studied railway stations were: *Convolvulus arvensis* L., *Erigeron canadensis* L., *Fallopia convolvulus* (L.) A. Löve, *Amaranthus hybridus* L., *Amorpha fruticosa* L. Individual plants of strongly invasive *Ambrosia artemisifolia* L. have been identified in the railway station in the Haskovo City.

15. **Stoyanov, P.,** Mladenov, R., Mileva, N., Todorov, K. (2022). Monitoring of Vascular plant species from the Southeastern part of Strandzha Natural Park, Bulgaria. *Ecologia Balkanica,* Special Edition 5: 7-15 Web of Science, Scopus Q4 SJR 0.137 (2021)

    Abstract. The study presents data on some invasive and conservation-significant species in two protected areas of the Strandzha Nature Park – Protected Area "Marina Reka" and Protected Area "Silistar". There were monitored the protected species *Pancratium maritimum*, *Calluna vulgaris*, *Daphne pontica*, *Ilex colchica*, *Rhododendron ponticum*, *Mespilus germanica,* and *Taxus baccata*, as well as an invasive species – *Amorpha fruticosa*, whose population borders that of *Pancratium maritimum*. The projective coverage of species in the sample areas and the total projective coverage of the vegetation have been studied also measures for their protection have been indicated.

16. Gyuzeleva, D., **Stoyanov, P.,** Bivolarska, A., Mladenov, R., Mladenova, Ts., Petkov, V., Todorov, K. (2022). Anatomical Investigation of *Marrubium friwaldskyanum* Boiss. and *Marrubium peregrinum* L. (Lamiaceae) from Bulgaria. *Ecologia Balkanica*, 14(1): 87-101 Web of Science, Scopus Q4 SJR 0.137 (2021)

    Abstract. The present study presents data on the anatomical characteristics of the leaf and stem in *Marrubium friwaldskyanum* and *Marrubium peregrinum*. The leaves in both species are amphistomatic with diacytic and anomocytic stomata. The leaf lamina shows differentiation of palisade and spongy tissue, and collateral vascular bundles. Non-glandular and glandular trichomes have been found on the epidermis of the leaves and stem. The non-glandular trichomes are unicellular linear and multicellular branched. Glandular trichomes are peltate, with a single-celled or two-celled structure. There is a difference between the two species in terms of the width of the epidermal cells of the stem, the thickness of the cortex, the thickness of the xylem and the phloem in the stem. The established differences in the anatomical features can be useful in future taxonomic studies within the genus *Marrubium*.

For Criterion G8.

PUBLISHED BOOK CHAPTER OR COLLECTIVE MONOGRAPH

1. Belkinova, D., Mladenov, R., Dimitrova-Dyulgerova, I., Teneva, I., **Stoyanov, P.,** Cheshmedjiev, S. Phytoplankton of the Stouden Kladenets Reservoir (Eastern Rhodope Mountains, Bulgaria). In: PU "Paisii Hilendarski", Jubilee Proceedings "Biological sciences for a better future", ISSN 1312-062X, University of Plovdiv Publishing House, 2012, 42-61.

    Abstract. The subject of the research was to determine the taxonomic composition, species richness and abundance of the summer phytoplankton in the Stouden Kladenets Reservo ir (the eastern Rhodope Mountains, Bulgaria). A total of 30 taxa were found, belonging to 6 Divisions: Cyanoprokaryota – 3, Chlorophyta – 14, Zygnemaphyta – 3, Chrysophyta – 1, Euglenophyta – 3 and Bacillariophyta – 6. The Divisions Chlorophyta (46,7%) and Bacillariophyta (20,0%) turned out to have the largest relative species richness in the fl oristic composition. The species' richness, density and biomass of the phytoplankton increase in the direction from the dam wall towards the tail end of the reservoir. On the basis of the species' composition, species richness and abundance of the phytoplankton, the Stouden Kladenets Reservoir aquatory (aquatic territory) can be divided into two zones, substan tially differing in water quality. The open reservoir water is oligotrophic, with signs of mesotrophy, whereas in the tail part there is strong eutrophication and algae bloom.

2. Bozov, P., **Stoyanov, P.,** Iliev, I., Mladenov, R., Vasileva, T. Antifeedant activity of natural neo-clerodane diterpenes, isolated from *Scutellaria alpina* L. and *Salvia splendens* Ker.-Gawl. and four synthetic derivatives, against *Leptinotarsa decemlineata* Say larvae. In: PU "Paisii

Hilendarski", Jubilee Proceedings "Biological sciences for a better future", ISSN 1312-062X, University of Plovdiv Publishing House, 2012, 186-194.

Abstract. Treatment of leaf disks with solvents of six neo-clerodane diterpenes isolated from *Scutellaria alpina* and *Salvia splendens* and of fi ve their synthetic derivatives, resalted in a signifi cant antifeedant activity against *Leptinotarsa decemlineata* larvae in choice and no-choice asseys. Relationship structure-activity and changes in the antifeedant activity associated with certain structural features of the compoundes are discussed. As a standard is used Scutecyprol A.

II. EXTENDED HABILITATION REFERENCE OF SCIENTIFIC WORK FOR CRITERION B4

CRITERION B4.

HABILITATION WORK - SCIENTIFIC PUBLICATIONS IN PUBLICATIONS THAT ARE REFERENCED AND INDEXED IN SCIENTIFIC INFORMATION DATABASES OF WEB OF SCIENCE AND SCOPUS:

№	Publication	Indexed/Referenced in:		Number of points
1.	Dimitrova-Dyulgerova, I., Marinov, Y., Mladenova, Ts., **Stoyanov, P.,** Stoyanova, A. (2019). Essential Oils Composition of the Endemic Bulgarian Plant Species *Micromeria frivaldszkyana* (Degen) Velen. (Lamiaceae). *Comptes rendus de l'Académie bulgare des Sciences*. 72(11): 1484-1491.	Web of Science Q4 IF 0.343 Scopus Q2 SJR 0.218		20
2.	Dinev, T., Rusenova, N., Tzanova, M., Grozeva, N., Gerdzhikova, M., **Stoyanov, P.,** Mladenova, Ts., Beev, G. (2020). Antimicrobial Potential of Methanolic Extracts from *Betonica bulgarica* Degen et Neič. (Lamiaceae). *Ecologia Balkanica*, 12(2): 165-174.	Web of Science Scopus Q4 SJR 0.144		12
3.	Mladenova, Ts., **Stoyanov, P.,** Todorov, K., Davcheva, D., Kirova, G., Deneva, T., Gyuzeleva, D., Mladenov, R., Bivolarska, A. (2021). Phytochemical and Biological Traits of Endemic *Betonica bulgarica* (Lamiaceae). *Separations*, 8(2): 11.	Web of Science Q2 IF 3.344 Scopus Q3 SJR 0.418		20
4.	Mladenova, Ts., **Stoyanov, P.,** Denev, P., Dimitrova, S., Katsarova, M., Teneva, D., Todorov, K., Bivolarska, A. (2021). Phytochemical composition, antioxidant and antimicrobial activity of the Balkan endemic *Micromeria frivaldszkyana* (Degen) Velen. (Lamiaceae). *Plants*, 10(4): 710.	Web of Science Q1 IF 4.658 Scopus Q1 SJR 0.765		25
5.	Mladenova, T., Batsalova, T., Dzhambazov, B., Mladenov, R., Teneva, I., **Stoyanov, P.,** Bivolarska, A. (2022). Antitumor and Immunomodulatory Properties of the Bulgarian Endemic Plant *Betonica bulgarica* Degen et Neič. (Lamiaceae). *Plants*, 11, 1689.	Web of Science Q1 IF 4.658 (2021) Scopus Q1 SJR 0.765(2021)		25
			Total by criterion B4	**102**

The extended habilitation reference includes 5 publications under criterion B4. Scientific publications are indexed in Web Science and Scopus and meet the requirement of the Regulations for the Development of Academic Staff of the Republic of Bulgaria for 100 points. These publications outline part of my research interests, focused in the field of Pharmaceutical Botany and are related to the establishment of various biological activities of plants from the Bulgarian flora, mostly with endemic status or those for which similar studies in the world literature are scarce. My personal contributions to these studies include: field work, collection, identification

and depositing of plants, preparation of samples for analysis, laboratory work to establish their activities, work on scientific publications. Part of the results related to the study of plant species are presented in scientific works outside this habilitation reference.

In recent years, there has been a growing trend worldwide for the use of herbal medicinal products and supplements for health care needs (Schuster, 2001; Ekor, 2014). This increased demand and interest in their use encourages new drug discoveries and developments (Ekor, 2014). Many of the active ingredients of new drugs are obtained from medicinal plants (Katiyar *et al*., 2012). According to the World Health Organisation, about 80% of all people in the world use medicinal plants, and their number are constantly increasing. Phytopreparations represent about 40% of all medicines and forms, and the medicinal plants needed for their preparation are about 20,000 species (Hardalova *et al*., 1994).

Phytochemical studies of rare and endemic plants are of scientific interest and thus assessment of biological features, distribution and natural resources is important for their conservation and sustainable use (Aneva *et al*., 2018). The object of phytochemical and biological research in this habilitation reference are the endemic plants *Micromeria frivaldszkyana* (Degen) Velen and *Betonica bulgarica* Degen & Nejč.

Betonica bulgarica Degen et Neičˇ. *(synonym Stachys bulgarica* Hayek*)* is an endemic plant belonging to the Lamiaceae family (Petrova, A., 2006). It grows on carbonate, gray and brown forest soils in the Central and Eastern Stara Planina and in the Thracian Plain (Petrova, A., 2006; Genova, E., 2011). There is considerable information on the general ecological, anatomical and morphological features of this endemic species found in Bulgaria (Grozeva *et al*., 2016). Although many authors include Betonica in the taxonomic rank of Stachys, morphological features and chemotaxonomic markers indicate that they form a separate genus (Koeva-Todorovska, 1979; Giuliani and Bini, 2012; Marin *et al*., 2004).

Betonica species are widely used in folk and more recently in official medicine (Bankova *et al*., 1999). They have anti-inflammatory, immunomodulatory, antimicrobial, anti-cancer and antioxidant properties (Khavani *et al*., 2005; Amirghofran *et al*., 2007; Salehi *et al*., 2007; Saeedi *et al*., 2008; Morteza-Semnani and Saeedi, 2009; Hajdari *et al*., 2010; Serbetci *et al*., 2010; Šliumpaitė *et al*., 2013; Jassbi *et al*., 2014; Tzanova *et al*., 2018). Nowadays, the more frequent use of plant extracts as natural food supplements and a safer alternative to antimicrobial agents leads to the need to investigate their antimicrobial activity (Mostafa *et al*., 2018). *Betonica bulgarica* is known to have high polyphenols and flavonoids content which have not only antioxidant but also antimicrobial activity (Bankova *et al*., 1999; Tzanova *et al*., 2018; Yakoub *et al*., 2018). In addition, many species of the genus *Betonica* (*Stachys*) have been found to be rich in essential oils that also have antimicrobial activity. (Skaltsa *et al*., 2003; Grujic-Jovanovic *et al*., 2004; Vundac *et al*., 2006; Salehi *et al*., 2007; Morteza-Semnani and Saeedi, 2009; Hajdari *et al*., 2011). As the chemical polymorphism of medicinal plants largely depends on various factors such as geographical conditions, collection time, vegetation phase, etc., the study of medicinal plants is an important part of plant research (Igwaran *et al*., 2017). Differences in the content of chemical constituents can lead to variations in the antimicrobial activity of cultivated plants, since many chemical constituents exhibit antimicrobial activity (Das *et al*., 2009).

It was found that the methanolic extracts of *Betonica bulgarica* from different places in Bulgaria showed moderate antibacterial activity and also demonstrated good antioxidant properties, which correlated with flavonoid and total phenolic content of the studied samples. The significant amount of sesquiterpenes, flavonoids (mainly rutin, quercetin, hispidulin) and other phenolic compounds (Tzanova *et al*., 2018) suggest that extracts and essential oils obtained from *Betonica bulgarica* may have a wider biological activity beyond the determined antibacterial and antioxidant properties and thus may induce beneficial effects on human health.

Although the medicinal properties of Lamiaceae species are probably due to their essential oils and flavonoids, in recent years there has been an increased interest in some micronutrients (Łozak *et al*., 2002), the presence of which could have a synergistic effect with these components (Kremer *et al*., 2012). Most trace elements are essential for higher plants, mainly in the composition of metalloenzymes and metalloproteins. They are also involved in a number of redox processes, photosynthesis processes, respiration, gene expression and regulation, protein synthesis and plant defense mechanisms (Stančić *et al*., 2016).

The genus *Micromeria* Bentham also belongs to the Lamiaceae family, which is represented by about 70 species, 21 of which are distributed in Europe (Tutin *et al*., 1972). A number of molecular and morphological studies show a close relationship between the genera Micromeria (section Pseudomelissa) and Clinopodium (Bräuchler *et al*., 2005; Bräuchler *et al*., 2008) and refer the members of the genus *Micromeria* to the genus *Clinopodium* (Bräuchler *et al*., 2008).

Several species of *Micromeria* have been reported to possess antirheumatic, antiseptic and CNS stimulant effects, and several polyphenolic compounds have been identified in them (Tabanca *et al*., 2001). Polyphenols reduce inflammatory mediators, leukocyte migration and stabilize endothelial cells, thereby participating in the pathogenesis of vascular disorders. Traditionally, species of the genus *Micromeria* have been used against heart diseases, respiratory diseases (asthma), headaches, colds, wounds, skin infections, as well as for insecticidal, herbicidal and culinary purposes. Representatives of the genus *Micromeria* have been reported to have various in vitro biological effects: antioxidant (Vladimir-Knežević *et al*., 2011), antimicrobial (Herken *et al*., 2012), anticholinesterase (Lee *et al*., 2009), anti-inflammatory and analgesic (Shehab *et al*., 2012). This is indicative of their great potential for use as herbs, medicines and nutritional supplements (Vladimir-Knežević *et al*., 2015).

In Bulgaria, the genus *Micromeria* is represented by four species: *Micromeria juliana* (L.) *Bentham ex Reichenb*., *Micromeria cristata* (Hampe) Griseb*., Micromeria dalmatica Bentham ssp. bulgarica* (Velen.) and *Micromeria frivaldszkyana* (Degen) Velen. *Micromeria frivaldszkyana* is a Bulgarian endemic, distributed mainly in the rocky areas of the Central and Eastern Stara Planina in Bulgaria. There are several studies on the anti-inflammatory and antimicrobial effects of *Micromeria* against some pathogens (Tabanca *et al*., 2001; Duru *et al*., 2004; Brahmi *et al*., 2017). Ali-Shtayeh et al. reported that the antimicrobial activity of extracts from various *Micromeria* species may be due to plant phenolic compounds (Ali-Shtayeh *et al*., 1997). However, the antimicrobial properties of *Micromeria frivaldszkyana* have been little studied. Previous studies reported only 2,2'-diphenylpicrylhydrazyl (DPPH) radical activity of *Micromeria frivaldszkyana* and TLC analysis of flavonoid profiles of four *Micromeria* species distributed in

Bulgaria [Nikolova *et al*., 2017). More detailed information on the phytometabolites and biological activity of *Micromeria frivaldszkyana* is still lacking.

MAIN SCIENTIFIC CONTRIBUTIONS

1. For the first time, the essential oils from the leaves and flowers of *Micromeria frivaldszkyana* were analyzed using the GC–MS method (gas chromatography–mass spectrometry). Oil yield during the flowering phase was 0.69% in flowers and 0.26% in leaves. In the essential oil of the flower, 30 components were identified, representing 98.45% of the total content, with the main components above 3% pulegone (45.05%), germacrene D (18.43%), limonene (9.68%), γ-elemene (8.77%) and p-menthone (6.33%). Twenty-four were the identified components in the leaf oil, representing 98.45% of the total content, with constituents above 3% being pulegone (42.61%), p-menthone (28.33%), germacrene D (8.31%), limonene (5.99%) , γ-elemene (4.56%) and menthol (3.51%). The study showed that *Micromeria frivaldszkyana* during flowering can be a good source for obtaining pure pulegone. [B4.1]
2. The antimicrobial activity of *Betonica bulgarica* extracts was determined by the agar diffusion method. *Betonica bulgarica* root extracts showed the highest antibacterial activity against Staphylococcus aureus and Bacillus cereus with large zones of inhibition. All extracts demonstrated low or no statistically significant activity against *Escherichia coli*. In general, extracts from the Ablanovo locality (in the Blue Stones Nature Park) exhibit the highest activity against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*. Extracts of leaves, flowers, stems and roots of *Betonica bulgarica* showed no or weak and statistically insignificant antifungal activity. [B4.2]
3. The composition of *Betonica bulgarica* essential oil was determined by GC–MS method (gas chromatography–mass spectrometry). A high concentration of sesquiterpene hydrocarbons was found, the main compounds of which are ß-caryophyllene (17.4%), germacrene D (9.9%) and β-bourbonene (6.7%). [B4.3]
4. The content of 15 trace elements in *Betonica bulgarica* was determined by the ICP–MS method (inductively coupled plasma mass spectrometry). Among the investigated microelements, the content of manganese (177.2 µg/g) and strontium (156.8 µg/g) is the highest. The distribution of the studied trace elements is in the following order: Mn > Sr > Fe > Ba > Si > Ti > Zn > Cu > Al > Rb > Ni > Pb > Mo > Cr > Cs. Except for Ba, all other elements are found more in the leaves and inflorescence than in the stem. [B4.3]
5. Two types of trichomes were found on the upper and lower epidermis of *Betonica bulgarica* leaves - covering and glandular. Integumentary trichomes are multicellular linear, unbranched, while glandular trichomes are thyroid, sessile, with a four-celled head. This type of glandular trichomes, according to the descriptions of Giuliani & Maleci Bini, belongs to type B, where the radiating cell has a two- or four-cell structure and is mainly characterized by a polysaccharide content. [B4.3]
6. The content and composition of sugars and organic acids in the aerial parts of *Micromeria frivaldszkyana* were determined. The highest percentage is glucose (2.77%), followed by

fructose (1.18%) and galactose (0.82%). The disaccharides sucrose and melibiose have also been identified. The total amount of sugars was calculated at 5499 mg/100 g. [B4.4]

7. The content and composition of organic acids in the aerial parts of *Micromeria frivaldszkyana* was determined. A total of eight organic acids were detected, with quinic acid having the highest content - 556.3 mg/100 g DW. Citric acid is also present in significant amounts - 341.5 mg/100 g DW. [B4.4]
8. The content of total polyphenols and total flavonoids in the aerial parts of *Micromeria frivaldszkyana* was determined. The highest amount is rosmarinic acid (20.40±1.97 mg/g), followed by chlorogenic acid (1.380±0.080 mg/g), traces of ferulic acid and the presence of the flavonoid aglycon - apigenin (0.12 ±0.01 mg/g) Apigenin has antimicrobial and antioxidant properties, suppresses the development and spread of cancer cells. [B4.4]
9. The antioxidant activity of *Micromeria frivaldszkyana* was investigated by six methods: 2,2'-diphenylpicrylhydrazyl (DPPH)-286.4 ± 10.43 mM TE/g, 2,2'azinobis(3)-ethylbenzthiazoline-6-sulfonic acid (ABTS) - 358.4 ± 10.4 mM TE/g, iron-reducing antioxidant power (FRAP) - 388.0 ± 32.4 mM TE/g, copper-reducing antioxidant capacity (CUPRAC) - 905.6 ± 19.2 mM TE/g, oxygen radical absorption capacity (ORAC) - 3250.5 ± 208.1 µmol TE/g and hydroxyl radical scavenging capacity (HORAC) - 306.1 ± 23.5 µmol GAE/g. [B4.4]
10. The *in vitro* antimicrobial activity of *Micromeria frivaldszkyana* against nine microorganisms (*Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Penicillium chrysogenum, Aspergillus niger and Rhizopus arrhizus*) was studied, but the extract showed only against *Listeria monocytogenes* ATCC 19111 with an inhibition zone diameter of 9 mm and a minimum inhibitory concentration (MIC) of 10 mg/mL. [B4.4]
11. The strongest cytotoxic effect and antitumor activity against HeLa cell lines was found for an extract from the inflorescences of *Betonica bulgarica*. In the FL cell line, a stronger toxic effect was induced only by *Betonica bulgarica* leaf extract. The *Betonica bulgarica* stem extract exhibited the strongest *in vitro* cytotoxic effect against mouse cell lines. [B4.5]

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