ENHANCED HABILLITATION REPORT FOR SCIENTIFIC CONTRIBUTIONS

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This report presents the most significant scientific contributions from research carried out after the acquisition of the academic position "associate professor"

Total number of publications - 80; outside competitions for acquisition of the scientific and educational degree "doctor" and the academic position "associate professor" – 51; on indicator V – 13 (Q1 - 3, Q2 - 8, Q3 - 2); on indicator G – 20 (Q1 - 5, Q2 - 4, Q3 - 7, Q4- 4)[1].

Phytochemical investigations have been important, as 80% of the world's population use medicinal plants for healing and prophylaxis. Phytochemistry has a long-standing, close relationship with traditional medicine lasting over 200 years. New technologies for botanical determination, modern methods of phytochemical analysis and evaluation of biological activity are used to develop new strategies for improving quality control of medicinal plants. Phytochemistry plays an important role as a link between botany and biological activity in many aspects of ensuring safe and effective traditional medicines for most people in the world. All of this, as well as the importance of natural substances for organic chemistry, are responsible for the intense development of the chemistry of natural substances throughout the world.

Bulgaria is a country with a remarkable biodiversity of medicinal plants and is one of Europe's largest exporters of herbs. Phytochemical investigations of herbs are traditional in our country as well as in IOCCP-BAS. The laboratory in which I work is the only one in Bulgaria conducting scientific research in the field of sesquiterpene lactone chemistry (reference to Scopus) and leading in the research of secondary metabolites in plants.

The scientific contributions presented in this competition are the result of **interdisciplinary research**, which can 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.

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 (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, ^{1}H -^{13}C HSQC, ^{1}H -^{13}C HMBC, NOESY, UV, IR and MS).

1.1. Secondary metabolites in the representatives of family Asteraceae:

Many representatives of family Asteraceae (chamomile, wormwood, tansy, yarrow, elecampane, etc.) are well known medicinal plants. Many species of this family have been found

[1] In **red** are pointed the publications on indicator V, and in **blue** - on indicator G

to produce sesquiterpene lactones predominately. Numerous studies have shown that these compounds are responsible for the healing properties of the plants (anti-inflammatory, anti-cancer, cytotoxic, antimalarial, etc.). On the other hand, sesquiterpene lactones are successfully used to solve chemotaxonomic problems. Sesquiterpene lactones are a great scientific challenge, as the diversity in their structures does not allow the use of common methods for their quantification. The lack of specific mass-fragmentation or chromophore requires in most cases, their isolation in pure form and structural characterization (mainly by NMR) before their use for quantitative measurements. Flavonoids and esters of quinic acid are other characteristic compounds for the plants in this family and responsible for their antioxidant properties. Plants of family Asteraceae have been and continue to be the main subject of my research.

Representatives of the genus Artemisia are the subject of numerous studies in the world but poorly studied in Bulgaria. In continuation of investigations of Artemisia species, growing in Bulgaria [7, 8, 9 and 13], *the chemical composition of another representative of the genus was studied.* ***Artemisia alba Turra*** *is a plant widely distributed in the southern parts of Europe. Its aerial parts have been traditionally utilized as a stomach digestive and tonic in the form of a decoction. A. alba is considered as an example of a problematic taxon because of its significant morphological variability. Literature survey on the chemical constituents of this species has also shown a great diversity among the populations of the plant.*

Thirteen compounds (11 sesquiterpenoids and 2 flavonoids) were isolated from the chloroform extract obtained from the aerial parts of ***A. alba.*** Three of them were found for the first time in the investigated species and **another 10** are new natural compounds. They are biogenetically related sesquiterpenoids with a germacrane, a eudesmane, a guaiane and an oplopane carbon skeleton and with a high degree of oxidation (OH, OOH, OAc and epoxy groups). These compounds could be described as derivatives of germacrene A and germacrene D. The isolation of only cyclic sesquiterpenoids distinguished our sample from the previously studied ones, which produced mainly acyclic sesquiterpenoids. The results of this study confirmed that *A. alba* did not produce sesquiterpene lactones, which are the characteristic components of many species of genus *Artemisia* as well as the intraspecific variability of this species [**57**].

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 UHPLC-PDA-MS analysis of the flower and leaf methanol extracts revealed similar phenolic profile and allowed identification of 13 additional compounds tentatively characterized by their chromatographic behaviour, UV patterns, and MS fragmentations. The presence of **five** of isolated compounds is reported for the first time in *A. alba*. The data obtained in our study were compared with previously reported data for the species from different localities. It has been found that the samples from the Balkan Peninsula produced both flavones and flavonol types of compounds in contrary to those from the Mediterranean countries, which contained only flavonol type. Therefore, this conclusion is important from chemical point of view and a deeper study of the flavonoids in *A. alba* in the future will contribute to the knowledge on the intraspecific variability of this taxonomically doubtful species [**74**].

The genus ***Inula*** *comprises more than 100 species widespread in Asia, Africa and Europe (Mediterranean region). The representatives of the genus are used in traditional medicine throughout the world, although more common in Traditional Chinese Medicine as expectorants, antitussive, antibacterial, antidiabetic, antitubercular, etc. remedies. It is noteworthy that only a few species Inula, mainly of Asian origin, have been subjected to detailed phytochemical and/or biological evaluations so far. Despite the small number of studied species more than 400 secondary metabolites have been isolated, some of them with relevant biological activities. There is a lack of data for the chemical constituents of the species of genus Inula, growing in Bulgaria (11 species and 3 varieties). Four species of the genus Inula were examined in our laboratory so far.*

The subendemic species ***I. aschersoniana var. aschersoniana*** was studied phytochemically for the first time. The aerial parts of *Inula aschersoniana* Janka var. *aschersoniana* afforded 3 sesquiterpene lactones, a flavonoid and **4** new pseudoguaiane-type sesquiterpenoids. The new compounds contained a cyclopropenone ring in their molecule. To the best of our knowledge, only few compounds have been isolated from natural sources so far – two acids and 4 sesquiterpene derivatives from *T. speciosa* and *I. lineariifolia* [**56**].

Alantolactone and isolanatolactone were isolated from the roots of elecampane (*Inula helenium* L.). This pair of structural isomers has been shown to display various pharmacologic activities such as hepatoprotective, anti-inflammatory, antitumor, antibacterial, antidematophytic, antifungal activities, etc. These two sesquiterpene lactones were further used as standards to assess the different methods for their extraction from the plant [**30**].

The flower heads of ***I. 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. It has been found a significant difference in the skeleton type of sesquiterpene lactones accumulated in European and Asian origins of the species. i. e. an existence of intraspecific variability. Thus, the domination of guaianolides and eudesmanolides or their seco-derivatives characterize European and Asian taxa, respectively [**64**].

Investigation of the chloroform extract obtained from the aerial parts of ***I. oculus-christi*** L. led to the identification of 6 new sesquiterpene lactones (1 eudesmanolide and 5 guaianolides), in addition to the known gaillardin, pulchellin E, pulchellin C and 3-O-palmitates of 16β-hydroxylupeol, 16β-hydroxy-β-amyrin, and faradiol. It was found that guaianolides were dominating and gaillardin was the principal one like in *I. oculus-christi* of other origins studied so far. On the other hand, pulchellin E was detected in *I. oculus-christi* from Montenegro, while pulchellin C − in the plant from Iran. Our results are consistent with the previous reports and enrich the knowledge on the lactone profile of *I. oculus-christi* with the newly identified components. The obtained results could be of chemotaxonomic interest [**69** and **73**].

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 [**66**].

Preliminary cytotoxicity study of extracts and fractions enriched in sesquiterpene lactones, flavonoids and phenolic acids from *I. oculus-christi* showed stronger impacts of studied extracts on cancer cells rather than on non-cancer cells. Fractions enriched in sesquiterpene lactones showed stronger cytotoxic effect against the cancerous A549 cell line. The extract, enriched in phenolic acids and flavonoids was cytotoxic only at higher concentrations and longer treatment without significant differences between cancerous and non-cancerous cells. These results are an additional conformation of the anti-tumour activity of sesquiterpene lactones and extracts from *I. oculus-christi* [63 and 77].

The Balkan endemic species ***Jurinea tzar-ferdinandii* Davidov** was studied for the first time. Phytochemical study allowed the identification of twenty-two components: 12 triterpenoids (alcohols, their 3-*O*-acetates and 3-*O*-palmitates), 6 sesquiterpene lactones and 4 flavones. The isolated sesquiterpene lactones were with germacrane, eudesmane and elemane carbon skeleton. All of them contained 4-hydroxymethacryloyl ester residue at C-8 and 12,6-*trans*-α-methylene-γ-lactone ring. The described above lactones were isolated for the first time from *Jurinea* species and the lack of any functional group at C-14 distinguished them from the lactones found so far in the genus *Jurinea,* all of which contained oxygenated substituent at this position. The literature survey revealed that these compounds have been previously detected in *Onopordon* and *Centaurea* genera. Further, all isolated flavonoids are 5,7-dihydroxy-6-methoxyflavones, differing in the substitution pattern of ring B: 4'-mono- and 3',4'-disubstituted with OH and/or OMe groups. It is worth to note, that the presence of flavonoids in *Jurinea* species is reported for the time now. These flavonoids have been described as components in some *Centaurea* and *Onopordon* species. *Onopordon* and *Centaurea* genera together with genus

Jurinea belong to tribe Cardueae (Asteraceae). Thus, based on the structural type of the isolated lactones *J. tzar-ferdinandii* probably occupies an intermediate position and supported biogenetic relationship of genus *Jurinea* and the other two genera. The described flavonoids support partially this hypothesis, but further studies are needed, in order to understand their chemotaxonomical role in this case [**75**].

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 half maximal inhibitory constants (IC_{50}) for the two enzymes were in the range of 28-150 μg/mL. The strongest inhibitory activity was found for the total chloroform extract and onopordopicrin [**75**].

The endemic species ***Centaurea davidovii***, was studied for the first time and 8α-(5'-hydroxyangeloyl)-salonitenolide was isolated and identified [**59**].

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. Guaianolides and germacranolides were found to be characteristic for sect. Hiorthia, to which belongs *A. rumelica*. Thus, the obtained results are in accordance with its place in the botanical taxonomical scheme of this genus [**35**].

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 [**53**].

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

"Herba Alchemillae" (Lady's mantle) is an aggregate of species collectively referred to as Lady's mantle. The drug possesses astringent, diuretic and antispasmodic properties, and is commonly used in traditional medicine as a cure for excessive menstruation and wounds. Different studies showed that the phenolic compounds (for example, tannins, and flavonoids) present in the plant are responsible for the pharmacological activity of Lady's mantle. The subjects of our investigations were 3 unexplored species from genus Alchemilla: ***A. mollis*** *(critically endangered species in Bulgaria according to the IUCN criteria) and the Bulgarian endemics –* ***A. jumrukczalica*** *(critically endangered) and* ***A. achtarowii*** *(endangered).*

The work-up of the EtOAc fraction obtained after partition of the total methanol extract from the aerial parts of the three *Alchemilla* species led to isolation of **24** compounds in total, **one** of them was found to be a new natural compound [**36**, **40** and **65**]. The isolated compounds were flavonoid glycosides of kaempferol and quercetin types mainly. The summarized literature data for the content of flavonoids in *Alchemilla* species showed that the group of studied species is too small part (less than 10) in comparison with the huge number of species in this genus (more than 300). Some of the isolated compounds have been previously detected in other species such as *A. speciosa*, *A. vulgaris* and *A. xanthochlora*, but the available data are insufficient for any chemotaxonomical conclusions.

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. It has been found that EtOAc-fractions obtained after partition of the total methanol extract were the most active radical scavengers. The newly discovered compound, gossypetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside showed relative good antiradical scavenging activity [**36**]. It was confirmed that quercetin glycosides were better radical scavengers than kaempferol-3-galactoside and catechin [**65**].

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

*The representatives of genus **Heracleum**, used as spices and herbs are rich source of secondary metabolites with diverse biological activity, but there is a lack of studies on the species distributed in Bulgaria.*

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. The leaf and fruit extracts of *H. angustisectum* showed the highest DPPH radical scavenging activity and TEAC, while the roots of *H. verticillatum* and *H.angustisectum* were the most effective inhibitors of acetylcholinesterase. All studied extracts were low inhibitors of *α*-amylase [79].

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 [68].

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* [44 and 52], *Inula britannica* [67], *Inula aschersoniana* [71], *Inula oculus-christi* [76]), Lamiaceae (*Sideritis scardica* [41, 48 and 50], *Panzeria lanata* [49] and *Thymus longedetatus* [80]) and Apiaceae (*Seseli rhodopeum* [42], *Seseli rigidum* [51]) 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. The knowledge about the essential oil profiles of the studied species was enriched. In addition, chemotaxonomic dependencies, correlations between chemical content and ecological conditions as well as assessment of the influence of the plant growth regulators on accumulation of certain components in *in vitro* cultures were looking for. 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. All samples were characterized by low contents of oxygenated mono- and sesquiterpenes (≤1.6 and 2.3%, resp.). Principal component analysis (PCA) and cluster analysis (CA) showed a significant variability in the chemical composition of the studied samples as well as a correlation between the oil profiles and the ecological conditions of the natural habitats of *S. scardica* [48].

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 [50].

The chemical composition of the volatile extract from aerial parts of ***Inula oculus-christi*** L. was studied by GC-MS. Forty-six chromatographic peaks representing 95.0% of the total oil were detected. The oil was characterized by relatively high concentration of oxygenated terpenes (51.1%), fatty acids (16.7%) and aromatic compounds (14.8%). 1,8-Cineole and hexadecanoic acid were the principal constituents. The obtained results were compare with the literature data for the volatiles in *I. oculus-christi* from Iran. To the best of our knowledge, this report is the only one, which describes essential oil components of *I. oculus-christi*. The chemical composition of the volatile extracts of Iranian and Bulgarian *I. oculus-christi* showed more

differences than similarities, analogously to their lactone profiles and revealed intraspecific variation, which is probably due to specific ecological conditions in the two countries and / or to genetic factors [76].

The separately distilled flowers and leaves essential oils of ***I. britannica*** L. were investigated using GC–FID and GC–MS and a total of 83 constituents were registered. The oils were rich in terpenoids (57.85% and 77.28%), of which sesquiterpenoids dominated. The main constituents of the essential oils were viridiflorol and himachalol followed by 6,10,14-trimethyl-2-pentadecanone, 13-tetradecanolide and 3-methyl-4-propyl-2,5-furandione. The obtained data compared with the only two published for plants, growing in Ukraine and China showed again significant differences, similar to those found for the sesquiterpene lactone content [67].

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 (in total 16.7%). Cluster analysis (CA) was used for determination of the relationship between the species in *Inula verbascifolia* aggregate. Three groups were determined according to CA: the first group was characterized by high content of alkanes and fatty acids; the second cluster contained almost equal amounts of oxygenated monoterpenes, sesquiterpene hydrocarbons and aromatic compounds; the third cluster contained primarily terpenoids (91.6%) [71].

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. The first oil type was characterized by a higher concentration of sesquiterpenoids, of which germacrene D was the principal component. The second oil type was characterized by an increase in oxygenated monoterpenes, of which irregular monoterpenoids (yomogi alcohol, artemisia alcohol and artemisia ketone) were in a significant percentage. For example, oxygenated monoterpenoids dominated over sesquiterpenoids in the control) and in media lacking cytokinin supplementation. Significant levels of irregular monoterpenoids were found in the control and in plants with high IBA supplementation. The addition of BA to the culture medium resulted in a drop of monoterpenoids and prevalence of sesquiterpenoids in the oils [44].

Unlike the aerials, underground parts of ***A. alba*** in all media were found to produce sesquiterpenoids mainly. This significant difference especially in the media with extensive root development raises the question of either a possible translocation of these components from the aerials towards the roots or of an existence of separate biogenetic pathways in these different organs [52].

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

These studies are a continuation of the research conducted in our laboratory for optimization of the conditions for the extraction of biologically active compounds from medicinal plants [23, 24 and 33].

Ultrasound-assisted extraction of alantolactone and isoalantolactone from elecampane roots (***I. helenium***) was studied. The effects of ethanol concentration, extraction time, temperature and number of extraction steps on the extraction yields of both sesquiterpene lactones were investigated. Gas chromatographic (GC) method was used for simultaneous determination of their contents in the corresponding extracts. 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. [30].

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.

4.1. Sesquiterpene lactones in *Arnica montana*

It is known, that sesquiterpene lactones, especially those of helenanlin type are responsible for the biological activity of A. montana. Therefore, the attempts for cultivation of A. montana are directed toward optimizing the conditions for improving the quality of the herb, i.e., increasing the content of helenalin type of sesquiterpene lactones.

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. It was found that *in vitro* propagated 2-year plants in full flowering stage accumulated higher amount of lactones in comparison to *in vivo* propagated 3-year plants and to the seed formation stage, respectively. Helenalins predominated in *in vivo* propagated 2-year or *in vitro* propagated 3-year plants. 2-Methylbutyrate (2MeBu) was the principal ester in the samples with prevalence of helenalins, while isobutyrate (iBu) was the major one in the samples with predominance of 11,13-dihydrohelenalins. The results revealed that the environmental conditions on Vitosha Mt. are more suitable for cultivation of A. montana giving higher content of lactones [**62**].

4.2. Secondary metabolites in *Centaurea davidovii*.

The endemic Centaurea davidovii Urum. is represented by only a few and very fragmented populations in the floristic region of Stara planina Mt. Its limited distribution requires some in situ and ex situ conservation actions. In collaboration with colleagues from IBER-BAS, in vitro cultures were initiated from a single seed of C. davidovii. Whole plantlets were regenerated and cultivated for several months on agar solidified nutrient media differing by their composition: basal MS medium, MS medium supplemented with plant growth regulators, and liquid MS medium. Plantlets were ex vitro adapted and successfully acclimated to open-air conditions.

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 highest concentrations of all tested secondary metabolites were detected in the leaves of the acclimated plants during mass flowering, and the content of the lactone reaching 56.2 mg/g DW, which was several times more than in the other leaf samples. 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 [**59**].

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 $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ using spectrophotometric methods. It was observed a correlation between total phenolic content and antiradical activity. The extract obtained from flowers of *A. alba* possessed better antiradical activity and higher content of phenolic compounds and flavonoids

than the extract obtained from leaves of the plant. [74]. Similar results were obtained in the study of *I. oculus-christi*, i. e. flowers were richer in these groups of compounds and better radical scavengers [66]. Methanol extracts from both leaves and flowers of *I. britannica* were with higher content of phenolic compounds and flavonoids and better anti-radical activity compared to the corresponding chloroform extracts [64].

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

As a part of the project of IBER-BAS for conservation and sustainable use of high-mountain protected, endemic and rare medicinal plants were carried out experiments for cultivation of 3 species of the genus Alchemilla. The effectiveness of these experiments was assessed by spectrophotometric determination of the total amount of flavonoids and tannins in the respective samples.

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 [47].

The cultivation *ex situ* of *A. mollis*, *A. achtarowii* and *A. jumrukczalica* in two experimental high-mountainous fields (Vitosha and West Rodope Mt) at an altitude similar to their natural distribution does not cause significant changes in the content of flavonoids and tannins in the aboveground mass. A more significant variation in the content of flavonoids and tannins is observed between the different age groups within a single species than between the plants grown in two distinctive physical-geographical regions. The highest average content of tannins and flavonoids in aboveground phytomass from both experimental stations was found in *A. jumrukczalica* followed by *A. mollis*. The study of separate plant parts constituting the aboveground phytomass shows that flavonoids are predominately accumulated in the basal leaves, and tannins – in flowering stems of the plants. *A. mollis* flowering stems produced more flavonoids and tannins in comparison with *A. achtarowii* and *A. jumrukczalica* [54].

4.7. Furanocoumarins in *Heracleum* species

^{1}H-NMR spectroscopy was used for qualitative and quantitative determination of furanocoumarins in 4 *Heracleum* species. The comparative ^{1}H-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 roots of all studied species were characterized by the presence of pimpinellin as the main component, while fruit extracts were differing in the principal furanocoumarin: bergapten and imperatorin in *H. sibiricum* and *H. verticillatum*, respectively, and byakangelicol - *H. angustisectum* and *H. ternatum*. Leaves of *H. ternatum* and *H. angustisectum* did not contain this class of secondary metabolites. Thus, the Bulgarian endemic *H. angustisectum* and *H. ternatum* showed similar furanocoumarin profile. The data obtained for the Balkan endemic *H. verticillatum* were in accordance of previous findings for this species of Bulgarian origin. It should be mention that the results obtained in our study correlated well with the chemotaxonomic classification of the species in genus *Heracleum* proposed recently by Ušjak et al. According to this classification, *H. verticillatum* should be separated from *H. sphondylium* group due to the very different furanocoumarin content of its fruits. 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 [79].

Research prospects for the next 5 years

Over the next 5 years, I will continue my research in the thematic areas I am working on now, namely:

- phytochemical investigations of medicinal, aromatic, endemic, *in vitro* cultivated and unexplored plants in order to detect new biologically active secondary metabolites (terpenoids and phenolic compounds) and search for chemotaxonomic and/or structure-biological activity relationships;
- use of modern methods of extraction and structural characterization of biologically active compounds from medicinal plants and from waste products from manufactures using aromatic and medicinal plants;
- development of methods for quantification of biologically active compounds in medicinal plants and / or standardization of products obtained from medicinal plants.

Part of these studies will be related to my participation in the IOCCP-BAS projects under the EC Horizon 2020 Program, the National Scientific Program "Healthy Foods for a Strong Bio-Economy and Quality of Life" and the Center for Competence for the sustainable utilization of bio-resources and waste of medicinal and aromatic plants for innovative bioactive products.

Scientific research will continue, both through active cooperation within the IOCCP and the Bulgarian scientific community (IBER-BAS, BF-SU, etc.), as well as through international collaborations with teams from the University of Belgrade (Serbia), from the Anatolian University (Turkey), University of Cairo (Egypt), the Czech Academy of Sciences and others.