

PhytoBalk

Bioassay-guided approach for the standardized biotechnological yield of phytopharmaceuticals of valuable Balkan medicinal plants

Co-funded by the Swiss National Science Foundation and Ministry of Education and Science within the Bulgarian-Swiss Research Programme (BSRP) 2013-2016.

Main purpose of the project

Ex situ conservation sustainable biotechnological utilization of medicinal and aromatic plants from the Balkan region



International scientific team:

- **Zurich University of Applied Sciences, Institute of Chemistry and Biotechnology, Wädenswil, Switzerland**

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- **Institute of Organic Chemistry with Centre of Phytochemistry, BAS**

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- **Institute of Biophysics and Biomedical Engineering, BAS**

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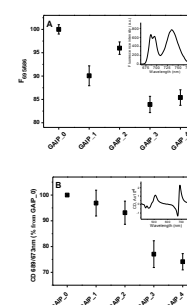
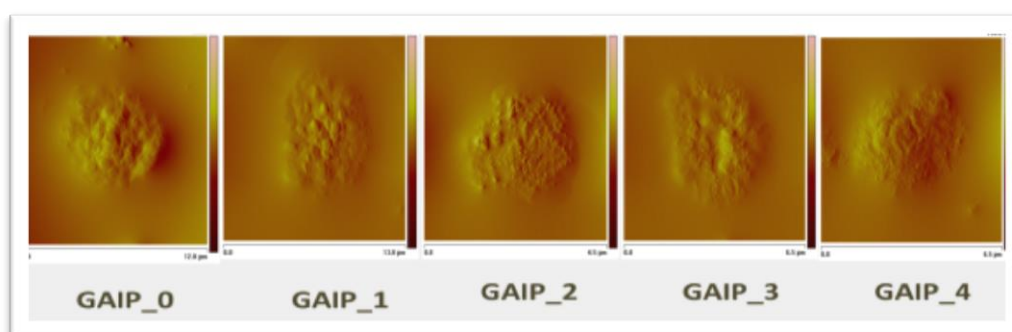
Main results:

- Biotechnological cultivation protocols for Balkan medicinal plant germ plasm - over 71 shoot accessions in solid medium, 8 genetically non-transformed roots and 8 suspension lines in liquid culture were developed. *In vitro* culture modifications were performed based on vitamin content, plant growth regulators, agar supplementations, active charcoal and light regime treatments.
- Isolation and identification of marker compounds from the wild collected material – phenolic acids, flavonoids, sesquiterpene lactones, coumarins. TLC confirmation of the presence of the components in the *in vitro* cultured material. HPLC, GC/MS quantification in the different *in vitro* derived samples.
- Essential oil distillation and GC GC/MS quantification in the *in vitro* cultured material.
- Characterization of the chloroplast morphology and PS II structure and function *in vitro*.
- Characterization of the electrophoretic profile and enzymatic activities *in vitro* (zymography and spectrophotometric assays).
- Rapid and Cost effective activity tests for Bioprocess control (Bioautography)

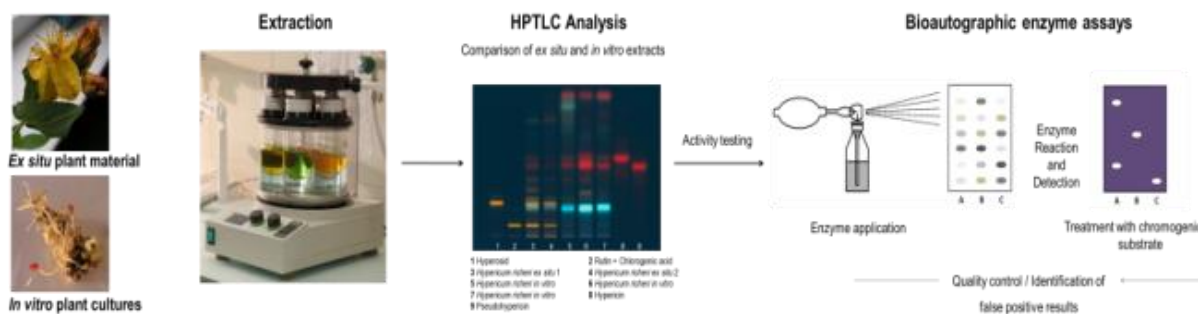
- Several active ingredients detected: antioxidants, inhibitors of Acetylcholinesterase, Xanthine Oxidase, Glucosidase



Extraction, purification and isolation of individual compounds.



Characterization of chloroplast morphology and Photosystem II functional characteristics.



Bioautography of obtained extracts and fractions of the *in vitro* cultured material.

Realized mobility within project implementation:

- Visits to the facilities of ZHAW of Bulgarian team members
- Training of young researchers at the facilities of ZHAW
- Attendance of Bulgarian and international conferences of team members and young researchers

Training of young people:

• Two diploma thesis works defended in Bulgaria:

- „Essential oils and flavonoid profile of *Artemisia alba* Turra” Master thesis, Viktorya Svetlinova Genova, Chimico-Technical and Metallurgy University, Faculty of Chemical technologies, scientific supervisors Assoc Prof Milka Todorova, Assoc Prof Antoaneta Trendafilova, IOCCP, BAS.
- „Structural organization of the photosynthetic apparatus in differentiated *in vitro* cultures of *Artemisia alba* Turra“ - Master thesis Bojidar Ivanov Enchev, Biotechnology, Scientific supervisor, Sashka Krumova, Institute of Biophysics and Biomedical Engineering, BAS. Consultant Prof. Stefka Taneva, from the same Institute.

• One diploma thesis work defended at Corvinus University, Budapest, Hungary:

- “Secondary metabolite production and antioxidant activity in *in vitro* cultured *Hypericum* species”
Master thesis in Agricultural Biotechnology, Rita Könye, Supervisors:
Dr. Zsuzsanna György, Assistant Professor, Genetics and Plant Breeding Department, Corvinus University of Budapest; Dr. Evelyn Wolfram, lecturer
Institute of Biotechnology, Phytopharmacy group, ZHAW, Wädenswil, Switzerland; Dr. Kalina Danova, Assist. Prof. Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia

• Young scientist scholarships for the support of initial stage researchers working in Bulgarian Academic Institutions of the World Federation of Scientists:

- MSci Petya Koleva – Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences.
- MSci Nia Petrova – Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences.

Publications:

- Krumova S, Motyka V, Dobrev P, Todorova M, Trendafilova A, Evstatieva L, Danova K (2013) Terpenoid profile of *Artemisia alba* is related to endogenous cytokinins *in vitro*. *Bulgarian Journal of Agricultural Science*, 19: 26–30 **IF**
- Todorova M, Trendafilova A, Danova K, Simmons L, Wolfram E, Meier B, Riedl R, Evstatieva L. (2015) Highly oxygenated sesquiterpenes in *Artemisia alba* Turra, *Phytochemistry*, 110:140-9. **IF**
- Taneva G, Markovska Y, Wolfram E, Danova K. (2014) Effect of plant growth regulators on growth patterns and enzymatic antioxidant activities in *Hypericum calycinum* shoot cultures, *Bulgarian Journal of Agricultural Sciences*, 20 (1) 46-50, P-ISSN 1310-0351 **IF**
- Koleva P., Wolfram E., Pedrussio S., Raynova Y., Evstatieva L., Danova K. (2015) *In vitro* culture development and polyphenolics production of *Artemisia alba* Turra. *J. Bio Sci. Biotechnol.* 2015, SE/ONLINE: 131-136
- Raynova Y, Markovska Y, Idakieva K, Wolfram E, Danova K. Relations between enzymatic and non-enzymatic antioxidant defence involved in polyphenolics production of *Artemisia alba in vitro*, proceedings of Seminar of Ecology, Union of Scientist, Bulgaria, Sofia 24-25, pp 134-138, ISBN 978-954-2961-75-8:
- Todorova M., Trendafilova A., Krumova S., Idakieva K., Genova V., Markovska Y., Raynova Y., Evstatieva L., Wolfram E., Danova K. (in print for 2015) Interdisciplinary interaction for the biotechnological development of Balkan medicinal plant species, Proceedings book of the Seminar of Ecology 2014, 24-25 April, Union of Scientists, Bulgaria, Section Biology. 95-98, Farago, ISBN: 979-853-476-132-4.

Book chapters:

- Danova K (2014) Biotechnological Utilization of the Indigenous Biosynthetic Capacity of Medicinal and Aromatic Plants: Experience in the Genera *Hypericum* *Pulsatilla* and Essential Oil Bearing *Artemisia alba* Characteristic for the Balkan Region. Chapter 14, In: J.N. Govil (Editor), *Recent Progress in Medicinal Plants Biotechnology and Genetic Engineering II* & Vol.39, Studium Press LLC, USA, p 355-392.
- Danova K (2015) Potential of the Balkan Flora as a Source of Prospective *Hypericum* Genotypes for the Conventional and Biotechnological Delivery of Phytopharmaceuticals. Chapter 2 in: *Hypericum: Botanical Sources, Medical Properties and Health Effects*. Howard R. Davis (Ed), Series Plant Science Research and Practices, Nova Science Publishers, USA, ISBN: 978-1-63482-701-0 pages 19-52

Over 40 contributions to scientific events.