

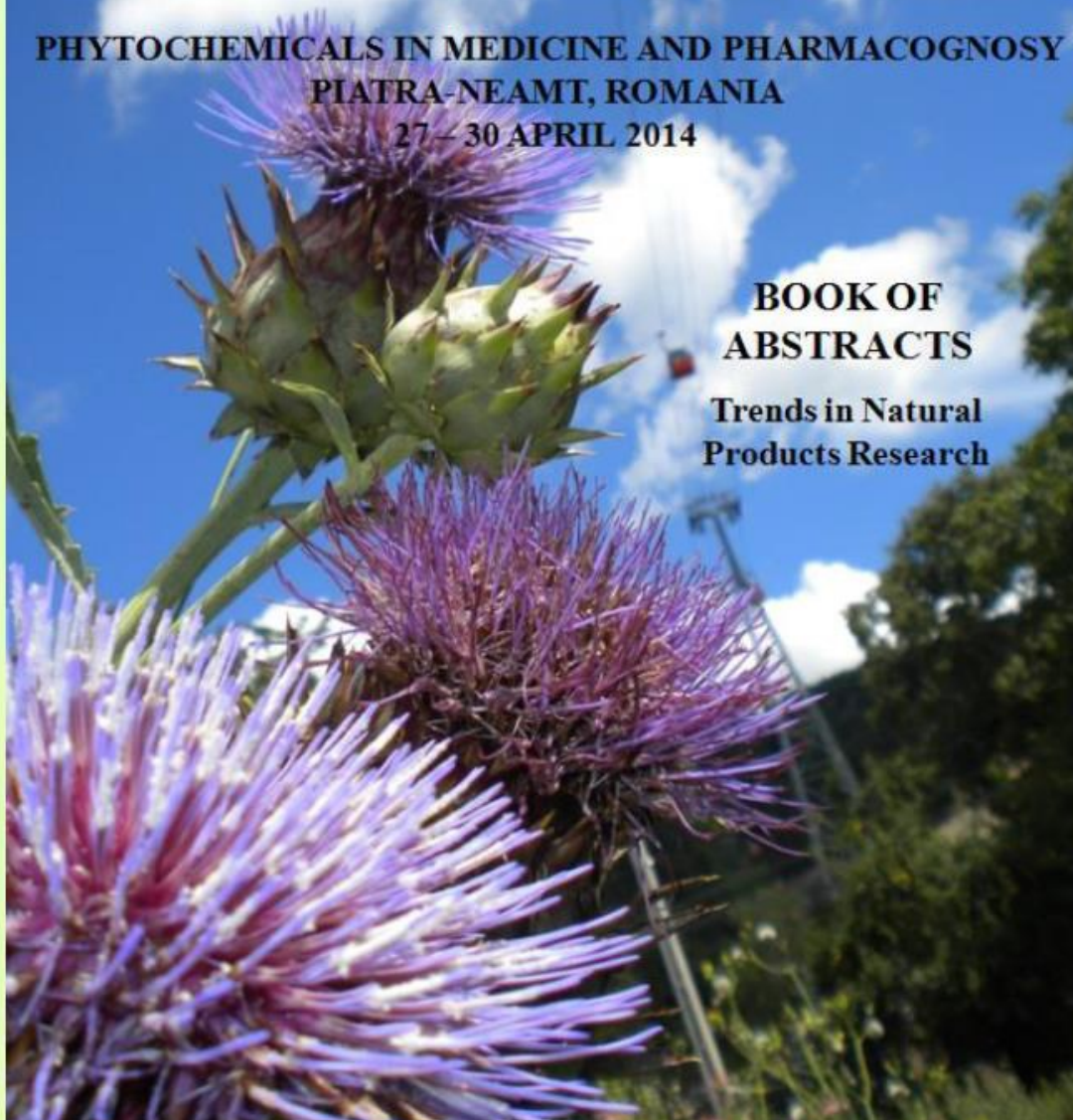
PHYTOCHEMICAL SOCIETY OF EUROPE MEETING



**PHYTOCHEMICALS IN MEDICINE AND PHARMACOGNOSY
PIATRA-NEAMT, ROMANIA
27 – 30 APRIL 2014**

**BOOK OF
ABSTRACTS**

**Trends in Natural
Products Research**



Bioautographic Xanthine Oxidase Assay: Combining phytochemical separation and activity assessment as a tool for phytopharmaceutical research on medicinal plants

Bräm Sarah^a, Meier B^a, Danova K^b, Wolfram E^a

^aZurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy

^bBulgarian Academy of Science, Institute of Organic Chemistry with
Centre of Phytochemistry, Sofia, Bulgaria

Xanthine oxidase (XO), a molybdenum containing flavoprotein, catalyses the oxidation of hypoxanthine and xanthine to uric acid under the formation of superoxide radicals and hydrogen peroxide. An overproduction of these reaction products in the human body is associated with diseases such as hyperuricemia, gout, hypertension, diabetes and different inflammatory diseases. Prospective long-term studies have demonstrated that the intake of Allopurinol as competitive xanthine oxidase inhibitor can cause a number of serious side effects. For this reason the research for specific xanthine oxidase debilitating compounds in Drug Discovery and thus also medicinal plants research is very important.

Bioautography offers a rapid and simple tool for screening of secondary metabolite profiles of medicinal plants by HPTLC combined with screening of potential health beneficial activities. The aim of this work has been to optimize and validate a bioautographic XO Inhibition assay described by Ramallo et al. [1].

To establish a reliable, reproducible and validate bioautographic XO assay, the concentrations of redox dye and substrate, enzyme activity as well as the buffer conditions were optimized using allopurinol as known inhibitory substance. Moreover, the assay procedure has been improved by using a low gelling temperature agarose and adjustment of incubation time and temperatures according to the XO thermal activity characteristics. XO inhibitory effects were visualised as white zones on a purple coloured thin layer chromatogram based on the reaction of superoxide radicals with nitroblue tetrazolium chloride. The visual detection limit of the competitive XO inhibitor allopurinol was 45.4ng. Extracts of *Camellia sinensis* and *Artemisia alba* showed also to contain constituents with XO inhibitory activity, that could be visually detected down to an applied amount of 10µg dry weight (dw) for *C. sinensis* extract and 100µg dw for *A. alba*. Since this assay uses superoxide radicals for the measurement of xanthine oxidase activity superoxide radical scavengers can also generate positive results on the HPTLC plate. However, such compounds can be differentiated from pure XO inhibitors by the direct measurement of uric acid by using a standard XO microtiterplate assay [2].

From the results it can be concluded, that the improved bioautographic Xanthine Oxidase inhibition assay is a rapid and valid research tool for assessment of active secondary metabolites from medicinal plants.

1. Ramallo I.A., Zacchino S.A., Furlan R.L.E. (2006). *Phytochemical Analysis*. 17: 15-19.

2. Havlika J., Gonzalez de la Huebra R., Hejtmankova K., Fernandez J., Simonova J., Melicha M., Rada V. (2010). *Journal of Ethnopharmacology*. 132: 461-465.

Acknowledgements: We thank Swiss National Science Foundation for the financial support of this work within the Bulgarian-Swiss Joint Research Project Project No. IZEBZ0_142989; DO2-1153

Terpenoid biogenesis in *Artemisia alba* is related to the structural organization of thylakoid membranes *in vitro*

Krumova Sashka^a, Tonya Andreeva^a, Todorova Milka^b, Trendafilova Antoaneta^b,
Wolfram Evelyn^c, Meier Beat^c, Danova Kalina^b

^aBulgarian Academy of Sciences, Institute of Biophysics and Biochemical Engineering, Sofia, Bulgaria; ^bBulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria; ^cZurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy, Wädenswil, Switzerland

Introduction: Terpenoids comprise over 30'000 different molecular structures isolated so far (1). Terpenoids such as plant hormones, photosynthetic pigments, signal transduction components, defensive molecules, and structural components of the plant cell wall play a major role for the survival of the plant organism (2). The pharmacological activity of many terpenoids imposes the challenge of better understanding the fundamental aspects affecting their biogenesis in the plant organism. The essential oil profile of the fragrant shrub *Artemisia alba* Turra has shown high variability in its terpenoid profile, attributed by different authors to environmental, climatic, as well as to genetic factors (3). Our previous research has shown that the terpenoid profile of the essential oils is strongly affected by the morphogenetic changes brought by auxin and cytokinin supplementation to the plant *in vitro* (4). As a part of the PhytoBalk project, financed by the Swiss National Science Foundation a complex study is ongoing for the better understanding of the fundamental aspects of terpenoid biogenesis in this plant.

Aim of the work: This work aims at the study of the structural and functional changes occurring in photosystem II of *A. alba* *in vitro* cultured plants, as well as in the area and height of thylakoid membranes in relation to the terpenoid profile of essential oils of the plant in the different model systems obtained by plant growth regulators treatment.

Methods: *In vitro* culturing techniques; micro-steam distillation; GC-MS identification and quantification of terpenoids in the essential oil; 77 fluorescence spectroscopy that gives information about the light energy utilization by the photosystems; circular dichroism spectroscopy that probes the macroorganization of the pigment-protein complexes and atomic force microscopy which probes the topography and morphology of the thylakoid membrane.

Results: Our data indicate that PGR treatment resulted in major structural changes in the thylakoid membranes — possibly altered macroorganization of the photosynthetic complexes and/or smaller photosystem II supercomplexes. Predominance of sesquiterpenoids in the essential oils was related to most prominent manifestation of this effect and was apparently related to a high diversity in the morphological parameters area and height of the thylakoid membranes, implying possible disturbance of chloroplast structure in this plant *in vitro* culture.

Conclusion: As it is known, the activity of the mevalonate-independent pathway of terpenoid biogenesis is involved in the biosynthesis of plastidic terpenoids, including monoterpenes, diterpenes and carotenoids. The predominance of sesquiterpenoids in the essential oils of *in vitro* cultured plants with impairment of chloroplast structure might possibly be explained with affecting the functionality of this pathway in *A. alba* *in vitro* model. The understanding and utilization of this effect might further be used as a practical tool for the delivery of essential oils with pre-determined chemical composition in this plant species.

1. De Luca V, St-Pierre B (2000) Trends Plant Sci. 5:168–173
2. McGarvey D, Croteau R (1995) Plant Cell 7:1015–1026
3. Radulović N, Blagojević P (2010) Natural Product Communications, 5: 1117–1122.
4. Danova K, Todorova M, Trendafilova A, Evstatieva L (2012) Natural Product Communications 7: 1–2.

Acknowledgements: We thank Swiss National Science Foundation for the financial support of this work. Project No. IZEBZ0_142989; DO2-1153

Biotechnological approaches for the targeted delivery of volatile and non-volatile biologically active secondary metabolites in *Artemisia alba* Turra

Danova Kalina^a, Todorova Milka^a, Trendofilova Antoaneta^a, Krumova Sashka^b, Peter Samuel^c, Bräm Sarah^c, Wolfram Evelyn^c

^aBulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria; ^bBulgarian Academy of Sciences, Institute of Biophysics and Biochemical Engineering, Sofia, Bulgaria; ^cZurich University of Applied Sciences, Institute of Biotechnology, Phytopharmacy, Wädenswil, Switzerland

Introduction: Decoctions of the aerials of *Artemisia alba* have been traditionally utilized as stomach digestive and tonic and its essential oil has been shown to possess spasmolytic and antimicrobial activities(1-3). However, the essential oil profile exhibits high variability depending on environmental and climatic impact, as well as to genetic factors (4). Also little is known on the non-volatile constituents present in the aerial parts of this plant. The main objective of the PhytoBalk project, financed by the Swiss National Science Foundation strives for the development of standardized biotechnological protocols, on the one hand to serve for *in vitro* conservation of valuable medicinal plants germplasm *ex situ*, and on the other to provide for the biotechnological delivery of pharmaceutically relevant raw plant material with standardized quality.

Aim of the work: The interplay between plant growth and development, production of essential oils, antioxidant polyphenolics, as well as the structural organization of the photosynthetic apparatus of the plant has been studied in a model system of exogenous plant growth regulators treatment *in vitro*.

Methods: Pharmacognostic methods combined with *in vitro* culturing techniques, subsequent analysis using adapted well established methods from Ph Eur HPLC and HPTLC methods as well as radical scavenging and antioxidant tests and preparative chromatographic isolation techniques, colorimetric assays and 77 K steady state fluorescent emission spectroscopy.

Results: Predominance of sesquiterpenoids in the essential oils was also related to stimulation of polyphenolic production and impairment of the structure and function of the photosynthetic apparatus in the plant. On the contrary, monoterpene dominated essential oils were produced by plants with lower polyphenolic productivity and was associated with a higher degree of aggregation of PS II peripheral antennae.

Conclusion: An *in vitro* system has been developed for the targeted production of raw plant material with the predominance of either monoterpenoids or sesquiterpenoids in the essential oils. Simultaneous stimulation of polyphenolics and biomass production has been achieved, as result of benzyl adenine treatment.

1. Rigat M, Bonet MA, Garcia S, Garnatje T, Vallès J. (2007). *Journal of Ethnopharmacology*, 113, 267-277
2. Ronse A, De Pooter HL. (1990). *Journal of Essential Oil Research*, 2, 237-242
3. Stojanovic G, Palic R, Mitrovic J. (2000). *Journal of Essential Oil Research*, 12, 621-624.
4. Radulović N, Blagojević P. (2010). *Natural Product Communications*, 5, 1117-1122.

Acknowledgements: We thank Swiss National Science Foundation for the financial support of this work. Project No. IZEBZ0_142989; DO2-1153