



BIO-ANTIOXIDANTS
2018

2nd International Conference
on Bio-antioxidants

2nd Young Scientists School
on Bio-antioxidants

BOOK OF ABSTRACTS

07—10 September 2018
Varna, Bulgaria

www.bio-antioxidants2018.com

Organization

Institutions

Institute of Organic Chemistry with Centre of Phytochemistry
Bulgarian Academy of Sciences, Sofia, Bulgaria

Co-organiser:

Bulgarian National Science Fund
Ministry of Education and Science

Co-organiser of the Young Scientists' School on Bio-antioxidants 2018

Foundation Evrika

Scientific Committee

Prof. DSc Veselina GADJEVA, Trakia University - Stara Zagora, BULGARIA
Prof. Dr. Pavlina DOLASHKA, Bulgarian Academy of Sciences, BULGARIA
Prof. Dr. Pavletta SHESTAKOVA, Bulgarian Academy of Sciences, BULGARIA
Prof. DSc Mona STANCHEVA, Medical University – Varna, BULGARIA
Prof. DSc Olga KASAIKINA, Russian Academy of Sciences, RUSSIA
Prof. DSc Aleksei TROFIMOV, Russian Academy of Sciences, RUSSIA
Prof. DSc Grzegorz LITWINIENKO, University of Warsaw, POLAND
Prof. DSc Ryszard AMAROWICZ, Polish Academy of Sciences, POLAND
Prof. Dr. Luciano SASO, Sapienza University of Rome, ITALY
Prof. DSc Virinder PARMAR, University of Delhi, INDIA
Prof. Dr. Mario FOTI, National Research Council, ITALY
Prof. Dr. Carlos BRAVO-DIAZ, University of Vigo, SPAIN

Organizing Committee

Prof. Dr. Vessela KANCHEVA, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, **Chairman**
Dr. Stefan DOCHEV, Ontime Alpha Seven Ltd., Viena, Austria, **Scientific Secretary**
Dr. Adriana SLAVOVA-KAZAKOVA, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, **Member**
Dr. Silvia ANGELOVA, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria, **Member**

Conference Organiser

Company for International Meetings Ltd.

Sponsors

Bioquochem, Spain
Metrohm
Shimadzu
Perkin Elmer

Saturday, 8 th September 2018			
Prof. Mario Foti, CNR Institute of Biomolecular Chemistry, Catania, Italy		Opening Plenary Lecture Mechanism of reactions of bio-antioxidants	9.00-9.45
Topic A: Oxidative Stress and Human Health Moderators: Prof. V. Gadjeva, Prof. R. Amorati			
PL1	Prof. Luciano Saso, Sapienza University, Rome, Italy	Pharmacological modulation of oxidative stress	9.45-10.30
	Coffee break		10.30-11.00
KL1	Prof. Veselina Gadjeva, Trakia Univ. St. Zagora, Bulgaria	Influence of combination antioxidants and UVB phototherapy on the level of oxidant/antioxidant status of vitiligo patients	11.00-11.30
IL1	Dr. Gaia Rocchitta, University of Sassari, Italy	Diet nutraceuticals: can the bioantioxidant content in food help in preventing neurodegenerative diseases?	11.30-11.45
Topic B: Natural Bio-antioxidants Moderators: Prof. L. Saso, Prof. V. Parmar			
PL2	Prof. Ricardo Amorati University of Bologna, Italy	Nano-antioxidants: where we are and where we are going	11.45-12.30
	Lunch break		12.30-14.00
KL2	Prof. Jan Stevens Oregon State University, USA	Polyphenols act as mild mitochondrial uncouplers	14.00-14.30
KL3	Dr. Kalina Danova, IOCCP, BAS, Bulgaria	Understanding plant-environmental interactions as a key tool to target antioxidant secondary metabolites production <i>in vitro</i>	14.30-15.00
Topics A and B: Short Oral Presentations Moderators: Prof. V. Parmar, Prof. J. Stevens			
OP1	Dr. Bozhil Robev, UMHAT "St. Ivan Rilski", Bulgaria	Impact of combination chemotherapy with irinotecan and cisplatin on plasma antioxidant capacity in lung cancer patients	15.00-15.15
OP2	Prof. Atanas Atanasov, Trakia Univ. St. Zagora, Bulgaria	Biologically active substances with antioxidant activity isolated from medical plant <i>Galega Officinalis L.</i>	15.15-15.30
	Dr. David Hevia, Bioquochem, Spain - sponsor	New method to measure antioxidant capacity in liquid samples	15.30-15.45
	Mr. Alexander Kirilov, Metrohm, Bulgaria - sponsor	Metrohm - high-precision chemical analysis tools	15.45-16.00
	Mr. Victor Vodenicharski, Shimadzu -sponsor	Bio-antioxidant analysis trough Shimadzu prism	16.00-16.15
OP3	Dr. MIMOZA Tzvetkova, MU, Sofia, Bulgaria	Comparative evaluation of RSA of cocoon extracts from different silkworm breeds	16.15-16.30
	Coffee break		16.30-17.00
	Poster session 2	Topics C, D, E and F	17.00-18.30
	Conference Dinner		20.00-22.30

B-KL3. UNDERSTANDING PLANT-ENVIRONMENTAL INTERACTIONS AS A KEY TOOL TO TARGET ANTIOXIDANT SECONDARY METABOLITES PRODUCTION *IN VITRO*

Kalina DANOVA,^{a*} Vaclav MOTYKA,^b Petre DOBREV^b

^a Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, BULGARIA

^b Institute of Experimental Botany, Czech Academy of Sciences, Rozvojová 263, 165 02 Prague 6 - Lysolaje, CZECH REPUBLIC

Secondary metabolites production in the plant organism is bound with its complex interrelations with the surrounding environment. In addition, developmental patterns and morphogenesis of the plant individual strongly affect its secondary metabolites content. The controlled environment of plant cell tissue and organ culture breeding makes plant biotechnology a flexible approach for targeting valuable phytopharmaceuticals through modification of growth conditions.

As a part of a broader program for biotechnological development of medicinal and aromatic plants, in the Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, *in vitro* collection of the essential oil bearing *Artemisia alba* was established. It was established that morphogenesis, growth and development of the plants were shown to be decisive for affecting terpenoid profile of its essential oils.¹ In continuation of this work, polyphenolics production in different types of *in vitro* cultured lines of the plant was studied. Leaf and root explants of sterile whole plant were used as starting material for liquid culture of non-differentiated suspensions and plant growth regulators treatments were applied on differentiated shoot cultures in order to modify their developmental patterns *in vitro*. Polyphenolic levels, hydrogen peroxide (as a marker of oxidative stress) and malondialdehyde (as a marker of lipid peroxidation) were determined spectrophotometrically.² The levels of endogenous phytohormones were quantified using an HPLC (Ultimate 3000, Dionex, U.S.A.) coupled to hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP, Applied Biosystems, U.S.A.) set in selected reaction monitoring mode by using a multilevel calibration graph with internal standards.³

The results indicated the distinctively different relations between production of antioxidant phenolics and flavonoids with the endogenous phytohormones in the non-differentiated and differentiated *A. alba in vitro* lines. In the differentiated shoot cultures disturbance of plant physiological status by suppression of normal root formation resulted in elevation of polyphenolics as a compensatory mechanism to overcome tissue culture induced stress, this effect being accompanied by elevation of jasmonic acid pool and suppression of bioactive cytokinins, abscisic and salicylic acid production. On the contrary, in non-differentiated suspensions, treatments which stimulated the polyphenolic production were associated with elevated levels of all three stress hormones (abscisic, jasmonic and salicylic acid). Interestingly, the initial lipid peroxidation levels of the explant source for suspension culture initiation seemed to be decisive for the physiological endogenous hormonal regulation, as well as the polyphenolics biosynthetic capacity of *A. alba* non-differentiated lines. Thus, the identification and correct interpretation of factors affecting secondary metabolite production in the plant organism are of crucial importance for the biotechnological delivery of secondary metabolites with antioxidant properties of medicinal and aromatic plants.

Keywords: *Artemisia alba*, endogenous hormones, growth and development, *in vitro* shoot cultures, *in vitro* suspensions, polyphenolics

References

1. K. Danova, V. Motyka, M. Todorova, A. Trendafilova, S. Krumova, P. Dobrev, T. Andreeva, Ts. Oreshkova, S. Taneva, L. Evstatieva, *J. Plant. Growth Regul.* **2018**, 37, 403–418.
2. P. Koleva, E. Wolfram, S. Pedrussio, Y. Raynova, L. Evstatieva, K. Danova, *J. Bio. Sci. Biotechnol.* **2015**, SE/ONLINE, 131-136
3. D. Djilianov, P. Dobrev, D. Moyankova, R. Vaňková R, D. Georgieva, S. Gajdošová, V. Motyka, *J. Plant Growth Regul.*, **2013**, 32, 564–574.

Acknowledgements: the Joint Scientific Research Project between the CAS and BAS (Reg. No. 17-17); the Czech Science Foundation (16-14649S).

B-PP14. PHYSIOLOGICAL FACTORS AFFECTING POLYPHENOLICS PRODUCTION OF *IN VITRO* CULTIVATED BALKAN ENDEMIC *SIDERITIS SCARDICA*

Kalina DANOVA,^a Ina ANEVA,^b Yuliana MARKOVSKA^c

^a Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, BULGARIA

^b Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, J. Gagarin 2 Str., 1113 Sofia, BULGARIA

^c Faculty of Biology, Sofia University "St. Kliment Ohridski", 8 Dragan Tsankov Blvd., 1164 Sofia, BULGARIA

The *Sideritis* genus (Lamiaceae) includes more than 150 species, distributed mainly in the Mediterranean region, the Balkans, the Iberian Peninsula, Central Europe and West Asia. The Balkan endemic *Sideritis scardica* is an aromatic medicinal plant, traditionally utilized as a pulmonary treatment, as well as anti-flu and wound healing remedy.¹ It has been established that presently there are less than 2 500 mature individuals in Bulgaria and over 250 in Serbia.² The plant has been determined with a Near Threatened status, with a decreasing current population trend³ and under the category "Endangered" in the Red List of Bulgarian vascular flora.⁴

Shoot cultures of the plant, collected from the Slavjanka Mountain in Bulgaria were initiated from sterile germinated seeds with the purpose of germplasm conservation and biotechnological delivery of secondary metabolites with antioxidant properties.

Plant growth regulators were applied in order to affect *in vitro* multiplication of the plant. Total phenolic and flavonoids content and hydrogen peroxide levels, low molecular antioxidants (ascorbate and glutathione), as well as antioxidant enzymes (ascorbate peroxidase, APx, EC 1.11.1.11; glutathione reductase, GR, EC 1.8.1.7; quaiacol peroxidase; GPOX, EC 1.11.1.7; catalase, CAT, EC 1.11.1.6 and phenylalanine amonilayase, PAL, EC 4.3.1.24) in the shoot cultures were assayed spectrophotometrically.

Supplementation of plant growth regulators (PGR) to the medium increased significantly the explants' survival rate, multiplication index and subculture period and stimulated the production of ascorbate and total phenolic and flavonoid compounds as compare with the PGR-free control. These treatments were also associated with elevation of endogenous hydrogen peroxide levels *in vitro*. The combination of 1.0 mg/l naphthaleneacetic acid with both 0.2 mg/l and 0.5 mg/l benzyl adenine were found to be most favorable for both highest polyphenolic production and optimal antioxidant enzymes functioning.

Hydrogen peroxide has a varied role in maintenance of the physiological status of the plant organism. On one hand its elevated levels are a consequence of the damaging impact of reactive oxygen species, but on the other it is also a messenger for induction of the plant's antioxidant defense and a mediator for establishment of resistance of the plant to environmental stress stimuli.

Further investigation of the qualitative characteristics of the produced polyphenolics is in process in order to assess the optimal conditions for the biotechnological delivery of potential phytopharmaceuticals of the Balkan endemic *S. scardica*.

Keywords: *Sideritis scardica*, shoot cultures, polyphenolics production, antioxidant enzymes, hydrogen peroxide *in vitro*

References

1. S. Ivancheva, B. Stantcheva, *J Ethnopharmacol.*, **2000**, 69, 165–172.
2. IUCN.2016. The IUCN Red List of Threatened Species, <http://www.iucnredlist.org/details/203271/>
3. S. Khela, *The IUCN Red List of Threatened Species*, **2013**: <http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T203271A2762714.en>.
4. A. Petrova, V. Vladimirov, *Phytol. Balc.*, **2009**, 15, 63–94.