



BOOK OF ABSTRACTS • KNJIGA ABSTRAKTA

*The International Bioscience Conference and the 6
th International PSU – UNS Bioscience Conference IBSC 2016*

Izdavač

*University of Novi Sad, Faculty of Sciences,
Trg Dositeja Obradovića 3. 21000 Novi Sad, Serbia*

Za izdavača

*Milica Pavkov-Hrvojević,
dean of the Faculty of Sciences*

Editors (urednici)

*Neda Mimica-Dukić,
Slobodanka Pajević and
Anamarija Mandić*

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Conference Report of IBSC 2016 Scientific Committee



On behalf of the Scientific and Organizing committees of The International Bioscience Conference and the 6th Joint international PSU-UNS Bioscience Conference 2016 between the Prince of Songkla University, Thailand and the University of Novi Sad, Republic of Serbia, we are honoured to host this Conference in Novi Sad, from 19-21 September 2016.

The International Bioscience Conferences are held every two years and represent the seal of a long-standing partnership between the Prince of Songkla University from Thailand and the University of Novi Sad from Serbia. IBSC 2016 is a joint endeavor to create a forum for networking, sharing ideas and achievements and hence contribute to the overall advancement in the complex and multifaceted field of bioscience. The chosen conference topics aim at providing knowledge, expertise, results and novel ideas in biosciences ranging from fundamental research, advancement in industrial research and strengthening the ties between industry and academia. IBSC 2016 puts a special emphasis on: Biodiversity and Environment; Physiology of living organism; Biotechnology, Bioengineering and Biosensing; Bioactive natural products-biochemistry and pharmacology and Agriculture, Food Science and Technology. The Scientific Program of the IBSC 2016 is delighted by the presence of Prof. Dr. Giacinto Bagetta from the Department of Pharmacy, Health Science and Nutrition, University of Calabria, Italy; Prof. Dr. Souwalak Phongpaichit, Faculty of Science, Department of Microbiology, Prince of Songkla University, Thailand; Prof. Dr. Marco Dalla Rosa, Alma Mater Studiorum, Università di Bologna, Italy; and Prof. Luciano Saso, Faculty of Pharmacy and Medicine, Sapienza University, Rome, Italy, who will immensely contribute to the conference as the key note speakers. We believe that together with the key notes, 13 other top quality invited speakers from around the world, overall number of 47 oral presentations, around 180 papers and 140 posters, this Conference can make a difference, enlighten and inspire all of us, especially the young scientists, to invest more in creating innovative approaches to solving the key challenges that the society is facing.

We look forward to welcoming around 200 international, regional and local scientists to Novi Sad, the city on the River Danube, the city of European history and hospitality, the cultural, economic, political and administrative centre of the Autonomous Province of Vojvodina in the north of Serbia, a modern university city, multicultural and multilingual urban centre, famous for its hospitality.

We wish to express sincere gratitude to all contributors, colleagues, sponsors and enthusiasts who provided selfless and generous support to make the Conference a success.

Prof. Dr. Neda Mimica Dukić

The IBSC 2016 Chairperson - President of the IBSC 2016 Scientific Committee

Committee

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2. Anamarija Mandić, vice president (University of Novi Sad, Serbia)
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Topic 1: Biodiversity and Environment

- Mirjana Miloradov (University of Novi Sad, Serbia)
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Topic 2: Physiology of Living Organisms

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- Lana Zorić (University of Novi Sad, Serbia)
- Nebojša Andrić (University of Novi Sad, Serbia)
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Topic 3: Biotechnology, Bioengineering and Biosensing

- Wilaiwan Chotigeat (Prince of Songkla University, Thailand)
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- Benjamas Cheirsilp (Prince of Songkla University, Thailand)
- Vladimir Crnojević (University of Novi Sad, Serbia)
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- Amornrat Phongdara (Prince of Songkla University, Thailand)
- Milan Martinov (University of Novi Sad, Serbia)

Topic 4: Bioactive Natural Products-Biochemistry and Pharmacology

- Wilawan Mahabusakum (Prince of Songkla University, Thailand)
- Neda Mimica Đukić (University of Novi Sad, Serbia)
- Guido Juergenliemk (University of Regensburg, Germany)
- Giacinto Bassetta (University of Calabria, Italy)
- Suda Chakthong (Prince of Songkla University, Thailand)
- Isidora Samojlik (University of Novi Sad, Serbia)

Topic 5: Agriculture, Food Science and Technology

- Dubravka Štajner (University of Novi Sad, Serbia)
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- Branko Čupina (University of Novi Sad, Serbia)
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- JanTuran, Director of Institute of Field and Vegetable Crops, Novi Sad, Serbia

GENERAL INFORMATION

PRESENTATION INFORMATION:

Registration is available at the Registration desk:

UNIVERSITY OF NOVI SAD (UNS) – RECTORATE BUILDING

- MONDAY, 19 SEPTEMBER, 14:00-18:00
- TUESDAY, 20 SEPTEMBER, 08:00-14:00

UNIVERSITY OF NOVI SAD (UNS) • FACULTY OF SCIENCES

Department of Mathematics/Informatics and Physics (ground floor) in front of Amphitheatre A1:

- TUESDAY, 20 SEPTEMBER, 14:00-19:00
- WEDNESDAY, 21 SEPTEMBER, 09:00-17:00

POSTER SET-UP

- MONDAY, 19 SEPTEMBER, 14:00-18:00
- TUESDAY, 20 SEPTEMBER, 08:00-14:00

Posters are displayed during the whole conference!

POSTER SIZE: A1 (594×841 mm) portrait format (stickers will be available in front of the posters);

T1, T2 and T3 poster sessions – Faculty of Sciences, Department of Mathematics/ Informatics and Physics (ground floor) in front of Amphitheatres A5 and A7;

T4 poster session – Faculty of Sciences, Department of Mathematics/Informatics and Physics lobby on the II floor;

T5 poster session – Faculty of Sciences, Department of Mathematics/Informatics and Physics (ground floor) in front of Amphitheatre A1.

ORAL PRESENTATIONS

Speakers are kindly invited to upload the presentation before their session.

CONTACT INFORMATION:

Help and support during the conference hours is available at the Registration desk:

Jelica Kurcanski
Conference Secretariat
Tel. +00381628053860



PanaComp – Zemlja čuda d.o.o.
Bul. Cara Lazara 96
21000 Novi Sad
Tel/Fax 021/ 466077
e-mail: mice@panacomp.net

**PROGRAM IBSC 2016
19–21 SEPTEMBER 2016**

MONDAY, 19 SEPTEMBER		
		Venue
14:00–18:00	REGISTRATION	University of Novi Sad (UNS)- Rectorate building
TUESDAY, 20 SEPTEMBER		
08:00–09:00	REGISTRATION	UNS-Rectorate building
09:00–09:30	OPENING CEREMONY	UNS-Rectorate building
09:00–09:05	Dušan Nikolić, Rector of the University of Novi Sad	Central
09:05–09:10	Milica Pavkov-Hrvojević, Dean of the Faculty of Sciences	Amphitheatre
09:10–09:15	Charoen Nakason, Vice president of PSU, Surat Thani Campus	
09:15–09:20	Wilaiwan Chotigeat, Dean of the Faculty of Sciences, PSU	
09:20–09:30	Neda Mimica-Dukić, President of the Scientific Committee	
09:30–10:30	COFFEE BREAK	UNS-Rectorate building
10:30–12:00	PLENARY LECTURES Chair: Neda Mimica-Dukić	Central Amphitheatre
10:30–11:15	Giacinto Bagetta , <i>Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, Rende, Italy</i> : Rational basis for translation of bergamot essential oil as neurotherapeutic	

11:15–12.00	Luciano Saso , <i>Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy</i> : Modulation of oxidative stress as a pharmacological strategy	
12.00–14 :00	LUNCH	UNS-Rectorate building
14.00–19: 00	LECTURE SESSIONS POSTER SESSIONS	Faculty of Sciences
	T1 T2, T3 T4	Amphitheatre A7 A5 A1
20:00	GALA DINNER	CUBO Restaurant

WEDNESDAY, 21 SEPTEMBER		
09:00–10:30	PLENARY LECTURES Chair: Wilawan Mahabusarakam	Faculty of Sciences
09:00–09:45	Souwalak Phongpaichit , <i>Natural Product Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand</i> : Bioactive fungal metabolites	A1
09:45–10:30	Marco Dalla Rosa , <i>Alma Mater Studiorum, Universita di Bologna, Italy</i> : Innovation in food science and technology as a social and environmental challenge	A1
10:30–12:00	COFFEE BREAK (POSTER SESSIONS)	Faculty of Sciences
12:00–14:00	LUNCH	Faculty of Sciences
14:00–17:30	LECTURE SESSIONS POSTER DISCUSSION	Faculty of Sciences
	T4 T5	Amphitheatre A1 A7
17:30–18.00	CLOSING REMARKS AND CLOSING CEREMONY	Faculty of Sciences

PROGRAM IBSC 2016
19–21 SEPTEMBER 2016

TUESDAY, 20 SEPTEMBER 2016

T1: BIODIVERSITY AND ENVIRONMENT

		Venue
14.00–16: 00	LECTURE SESSIONS	Faculty of Sciences
T1 14:00-15:20	INVITED LECTURES Chair: Sara Bumrungsri	Amphitheatre A7
14:00-14:20	T1– IL–1: Sara Bumrungsri , <i>Department of Biology, Prince of Songkla University, Hat Yai, Thailand: Ecosystem services of bats in Thailand, a case study</i>	
14:20-14:40	T1–IL–2: Snežana Radenković , <i>Department of Biology and Ecology, Faculty of Sciences, Novi Sad, Serbia: Diversity and conservation of hoverflies (Insecta: Diptera: Syrphidae) in Southeast Europe</i>	
14:40-15:00	T1–IL–3: Anchana Prathep , <i>Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand: Sea-grass bed as a carbon sink in Ranong Biosphere Reserve and Trang-Haad Chao Maimarin National Park: An important role of Seagrass</i>	
15:00-15:20	T1–IL–4: Milivoje Krvac , <i>Institute for Nature Conservation of Serbia, Belgrade, Serbia: Exploitation of threatened non – CITES and new discovered reptile species</i>	
T1 15:20-16:00	ORAL PRESENTATIONS Chair: Mirjana Vojinović Miloradov	Amphitheatre A7
15:20-15:35	T1–O–1: Mirjana Vojinović Miloradov , <i>University Of Novi Sad, Faculty Of Technical Sciences, Department of Power, Electronic and Telecommunication Engineering, Novi Sad, Serbia: Determination of key physico-chemical parameters of water bodies quality by fiber optic sensors (FOS)</i>	
15:35-15:50	T1–O–2: Maja Novković , <i>University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia: Being smart in a multi-stressed world - predicting the stress impact on <i>Lemna minor</i> L., <i>Ceratophyllum demersum</i> L. and <i>Mentha aquatica</i> L. along the Danube river using ANN trained algorithms</i>	
15:50-16:00	DISCUSSION	

16:00–17:30	COFFEE BREAK (POSTER SESSIONS)	Faculty of Sciences
T1 17:30-19:00	ORAL PRESENTATIONS Chair: Anchana Prathep	Amphitheatre A7
17:30-17:45	T1–O–3: Dragana Čučak , <i>University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology</i> : Microbiological water quality of selected surface waters in Vojvodina	
17:45-18:00	T1–O–4: Maja Lončarski , <i>University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia</i> : Removal of selected pesticides by magnetic and/or macroporous ion exchange resins	
18:00-18:15	T1–O–5: Milica Stanković , <i>Seaweed and Seagrass Research Unit, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand</i> : Seagrass biomass mapping techniques in Andaman coast of Thailand	
18:15-18:30	T1–O–6: Miljan Šunjević , <i>University of Novi Sad, Faculty of Technical Sciences, Department of Architecture and Urbanism, Novi Sad, Serbia</i> : Influence of urbanisation of Fruška Gora on environment and biodiversity	
18:30-18:45	T1–O–7: Marko Djurakić , <i>University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Novi Sad, Serbia</i> : Tea with Godot – the emergence of the phylomorphogeography as a conceptually valid method for the subspecies inference	
18:45-19:00	T1–O–8: Vladimir Nikolić , <i>Institute for Nature Conservation of Serbia, Belgrade, Serbia</i> : Forest reserves in Serbia - the status, distribution and synecology	
19:00-19:15	DISCUSSION	
20:00	GALA DINNER	CUBO Restaurant

PROGRAM IBSC 2016
19–21 SEPTEMBER 2016

TUESDAY, 20 SEPTEMBER 2016

T2: PHYSIOLOGY OF LIVING ORGANISMS
T3: BIOTECHNOLOGY, BIOENGINEERING AND BIOSENSING

		Venue
T2 14:00-14:20	INVITED LECTURES Chair: Slobodanka Pajević	Amphitheatre A5
14:00-14:20	T2-IL-1: Nebojša Andrić , <i>University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia: Endocrine disruptors and reproduction: mechanisms of action in steroid hormone-producing cells</i>	
T2 14:20-15:05	ORAL PRESENTATIONS Chair: Nebojša Andrić	Amphitheatre A5
14:20-14:35	T2-O-1: Bojana Stanić , <i>University of Novi Sad, Faculty of Technical Sciences, Department of Environmental Engineering and Occupational Safety and Health, Novi Sad, Serbia: Establishment of cell-based ELISA for quantitative measurement of environmental chemical-induced ERK1/2 activation in HepG2 cells</i>	
14:35-14:50	T2-O-2: Tanja Bulat , <i>Department of Pediatrics, Medical University of Vienna, Vienna, Austria: Transcriptomic and proteomic analysis of Arion vulgaris – proteins for probably successful survival strategies?</i>	
14:50-15:05	T2-O-3: Milan Borišev , <i>University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia: Daily dynamics of beech photosynthetic parameters is significantly disturbed by periodical drought and specific microhabitat conditions</i>	
T3 15:10-16:00	INVITED LECTURES Chair: Wilaiwan Chotigeat	Amphitheatre A5
15:10-15:30	T3-IL-1: Wilaiwan Chotigeat , <i>Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand, Center for Genomics and Bioinformatics Research Faculty of Science, Prince of Songkla University, Hat Yai, Thailand: Extra-function of ribosomal protein L 10a</i>	
15:30-15:50	T3-IL-2: Vladimir Crnojević , <i>BioSense Institute, University of Novi Sad, Novi Sad, Serbia: Biosense institute and the future of agriculture</i>	
15:50-16:00	DISCUSSION	

COFFEE BREAK (POSTER SESSIONS)		Faculty of Sciences
T3 17:30-18:45	ORAL PRESENTATIONS Chair: Ivana Kovačić	Amphitheatre A5
17:30-17:45	T3-O-1: Phutita Wongwaiyut , <i>Molecular Ecology and Evolution Research Unit, Department of Biology, Prince of Songkla University, Hat Yai, Thailand: The RNAi machinery of Col- lembola</i>	
17:45-18:00	T3-O-2: Darjana Ivetić , <i>Faculty of Technology, University of Novi Sad, Bul. Cara Lazara 1, 21 000 Novi Sad, Serbia: Determination of some elements for techno-economic enzymatic hydrolysis of cellulose in hydrothermally pretreated sugar beet shreds</i>	
18:00-18:15	T3-O-3: Ida Zahović , <i>Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia: Xanthan biosynthesis in laboratory bioreactor on effluents from white wine production discussion</i>	
18:15-18:30	T3-O-4: Ivana Kovačić , <i>University of Novi Sad, Faculty of Technical Sciences, Novi Sad, Serbia: Biomechanical and biomimetical parametrization of trunk-dominated trees</i>	
18:30-18:45	DISCUSSION	
20:00	GALA DINNER	CUBO Restaurant

PROGRAM IBSC 2016
19–21 SEPTEMBER 2016

TUESDAY AND WEDNESDAY, 20/21 SEPTEMBER 2016

T4: BIOACTIVE NATURAL PRODUCTS-BIOCHEMISTRY AND PHARMACOLOGY

T4 14:00-14:40	INVITED LECTURES Chair: Souwalak Phongpaichit	Amphitheatre A1
14:00-14:20	T4-IL-1: Wilawan Mahabusarakam, Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand: <i>Garcinia dulcis</i> Kurz: secondary metabolites and biological activity	
14:20-14:40	T4-IL-2: Olga Tzakou, Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece: Essential oils as green pesticide	
T4 14:40-16:00	T4-ORAL PRESENTATIONS Chair: Ivana Beara	Amphitheatre A1
14:40-14:55	T4-O-BB-5: Emilija Svirčev, University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia. Phenolic constituents and <i>in vitro</i> antioxidant and anti-inflammatory activities of different Rumex species	
14:55-15:10	T4-O-P-2: Isidora Samojlik, University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology, Novi Sad, Serbia: <i>In vitro</i> and <i>in vivo</i> antioxidant potential of essential oil of aniseed (<i>Pimpinella anisum</i> L. Apiaceae)	
15:10-15:25	T4-O-BB-3: Sanja Živanović, Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain: Title multi-level strategy for analysis of bioactive drug conformations	
15:25-15:40	T4-O-BB-4: Tanja Šarenac, University of Novi Sad, Faculty of Medicine, Department of Pharmacology, Toxicology and Clinical Pharmacology, Novi Sad, Serbia: Synthesis of 3-oxo bile acids: selected products in the biomedical research and their study by FT-IR spectroscopy	
15:40-16:00	DISCUSSION	

16:00–17:30	COFFEE BREAK (POSTER SESSION)	Faculty of Sciences
T4 17:30-18:45	T4-ORAL PRESENTATIONS Chair: Nataša Simin	Amphitheatre A1
17:30-17:45	T4-O-BB-7: Marina Milenković , <i>Department of Microbiology and Immunology, University of Belgrade-Faculty of Pharmacy, Belgrade, Serbia: Antimicrobial activity of essential oils of <i>Calamintha</i> Mill. species (<i>Lamiaceae</i>)</i>	
17:45-18:00	T4-O-BB-6: Dragana Čučak , <i>University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia: Antimicrobial activity of extracts obtained by supercritical extraction of yarrow (<i>Achillea millefolium</i>) and rose hip (<i>Rosa canina</i>) waste produced in filter tea factory</i>	
18:00-18:15	T4-O-BB-8: Rebeka Jójárt , <i>Department of Organic Chemistry, University of Szeged, Szeged, Hungary: Synthesis and 17β-HSD1 inhibition of novel 2- or 4-substituted 13α-estrone derivatives</i>	
18:15-18:30	T4-O-BB- 11: Diandra Pintać , <i>University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia.; <i>Vitis vinifera</i> leaf extracts – phenolic profile, antioxidant and neuroprotective properties</i>	
18:30-18:45	DISCUSSION	
20:00	GALA DINNER	CUBO Restaurant

WEDNESDAY 21 SEPTEMBER

14:00 – 16:00	LECTURE SESSIONS	Faculty of Sciences
T4	T4-INVITED SPEAKERS	Amphitheatre A1
14:00-14:40	Chair: Olga Tzakou	
14:00-14:20	T4-IL-3: Juraithip Wungsintaweekul , <i>Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Thailand: Cyclooxygenases and inducible nitric oxide synthase inhibitory activities of diterpenes from <i>Croton stellatopilosus</i></i>	
14:20-14:40	T4-IL-4: Nongyao Sawangjaroen ; <i>Department of Microbiology, Faculty of Science, and Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Thailand: <i>Coptosapelta flavescens</i> Korth. as a potential anti-giardial compounds source</i>	
14:40-16:10	T4-ORAL PRESENTATIONS	Amphitheatre A1
14:40-14:55	T4-O-P-1: Chaweewan Jansakul , <i>Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat-Yai, Thailand: Effects of short-term chronic lard- or palm oil consumption on vascular functions in middle-aged male rats</i>	
14:55-15:10	T4-O-BB-2: Khrongkhwan Thammatinna , <i>Molecular Ecology and Evolution Research Unit, Department of Biology, Prince of Songkla University, Hat Yai, Thailand: Immunity genes and antimicrobial proteins in collembola, <i>Folsomia candida</i></i>	
15:10-15:25	T4-O-BB-1: Ivan Ivani , <i>Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain: Development of a new force field for molecular dynamics simulations of DNA molecules</i>	
15:25-15:40	T4-O-BB-10: Srđan Bjedov , <i>University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Novi Sad, Serbia: Synthesis and biological activity of bile acid ethylidene amides and oxazolines</i>	
15:40-15:55	T4-O-BB-5: Biljana Božin , <i>University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia: Chemical diversity of primary volatile compounds in garlics (<i>Allium l.</i>, sect. <i>Allium</i>, <i>Alliaceae</i>)</i>	
15:55-16:10	DISCUSSION	
16:10–17:30	COFFEE BREAK AND POSTER DISCUSSION	Faculty of Sciences
17:30–18.00	CLOSING REMARKS AND CLOSING CEREMONY	Faculty of Sciences

**PROGRAM IBSC 2016
19–21 SEPTEMBER 2016**

WEDNESDAY 21 SEPTEMBER 2016

T5: AGRICULTURE, FOOD SCIENCE AND TECHNOLOGY

		Venue
14:00 – 16:10	LECTURE SESSIONS	Faculty of Sciences
T5 14:00-14:20	INVITED SPEAKERS Chair: Anamarija Mandić	Amphitheatre A7
14:00-14:20	T5–IL–1: Franc Bavec , <i>University of Maribor, Faculty of Agriculture and Life Sciences, Hoče/Maribor, Slovenia: Situation and trends in organic food production</i>	
14:20-14:40	T5–IL–2: Charassri Nualsri , <i>Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand: Selection of rubber clones for the white root (<i>Rigidoporus microporus</i> (klotzsch) Imazeki) disease tolerance</i>	
T5 14:40-16:00	ORAL PRESENTATIONS Chair: Ana Marjanović Jeromela	Amphitheatre A7
14:40-14:55	T5–O–1: Ana Marjanović Jeromela , <i>Institute of Field and Vegetable Crops, Novi Sad, Serbia: The achievements in rapeseed breeding - a result of applying mendelian rules</i>	
14:55-15:10	T5–O–2: Devappa Venkatappa , <i>Department of Plant Pathology, College of Horticulture, UHS Campus GKVK, Bengaluru, India: Bio-efficacy of curzate m8 (cymoxanil 8% + mancozeb 64%) against late blight (<i>Phytophthora infestans</i>) of tomato in Karnataka</i>	
15:10-15:25	T5–O–3: Maja Čavić , <i>University of Novi Sad Faculty of Technical Sciences, Novi Sad, Serbia: Optimal synthesis of the sower for the precise organic agriculture</i>	
15:25-15:40	T5–O–4: Ivan Knežević , <i>University of Novi Sad Faculty of Technical Sciences, Novi Sad, Serbia: Synthesis of the mechanism for configuration change of the seedbed cultivator</i>	
15.40-15:55	T5–O–5: Maja Manojlović , <i>University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia: Trace elements and pesticide residues in soils on organic and conventional farms in Vojvodina, Serbia</i>	
15:55-16:10	T5–O–6: Goran Kitić , <i>BioSense Institute, University of Novi Sad, Novi Sad, Serbia: Miniature soil moisture sensor that does not require calibration</i>	
16: 00–17: 30	COFFEE BREAK AND POSTER DISCUSSION	Faculty of Sciences
17:30 – 18.00	CLOSING REMARKS AND CLOSING CEREMONY	Faculty of Sciences A1



ABSTRACTS
PLENARY LECTURES

Professor Dr. Marco Dalla Rossa

Alma Mater Studiorum, Università di Bologna, Italy



Professor Dr. M. Dalla Rosa is a full Professor at Alma Mater Studiorum, Università di Bologna, Italy. He was born in Trento (Italy) in December 1955. In 1979, he graduated in Agricultural Science at the University of Bologna, doing a research work on Processing of Kiwifruit. From graduation until now he has been fully dedicated to the research work and academic activities. In 1984 he won a research fellowship to carry out a research period at the CSIRO, Sydney, which he implemented in 1985 working for four months as a visiting scientist at CSIRO and collaborating with Dr. R. J. Steele and Dr. R. Duckworth. From 1986 to 1998, he was a scientist in charge of several national research projects on quality properties of fruit and animal products. From 1990 to 1992, after winning a race examination, he worked as a university researcher (permanent position) in the field of the Food Technology at the University of Udine. From 1992 to 2000 he was an Associate Professor of Food Technology at The University of Udine. In 2000 he won the full professorship at the University of Bologna and since November 2000 he has been working as a Full Professor of Food Technology at the Degree Course of Food Science and Technology of Cesena (Bologna University). Professor Dalla Rosa was a President of the Degree Course Committee on Food science and Technology at University of Bologna, site of Cesena from 2004 to 2011. From 2010 to 2011 scientist responsible of the Operative Unit Food processing, consumption and health of the CIRI - Agrifood (Interdepartmental Centre of Industrial Agrifood Research) in the frame of the Emilia Romagna Region High Technology Platforms. In December 2011 he was elected the Director of the Interdepartmental Centre for the Agri-Food Industrial Research in the frame of the agreement between Emilia Romagna Region and University of Bologna. In March 2015 he was re-elected for the following three years.

Professor M. Dalla Rosa is author or co-author of more than 250 scientific publications or congress communications. Bibliometrics indices: H-index: 27 (Google scholar), since 2011: 20 (Google scholar); H-Index 21 (Scopus); i10-index (Google scholar) 61, since 2011: 46. Citations: 3204, since 2011: 1785 (Google scholar), 1659 (Scopus). Prof. Dalla Rosa is Co-inventor of the European Patent N. 14732953.6-1358 "Food Preparation with mushrooms and making process".

Besides research, Professor Dalla Rosa is a regularly appointed referee for high impact factor journals, a member of various editorial and advisory boards. He holds visiting professorships at Far Eastern Federal University (Vladivostok, RUS) and Kaunas College (Kaunas, Lithuania).

Professor Dalla Rosa has been in charge of many research projects at regional, national and European level.

He received the ISEKI Food Association Academy Award in 2014.

Full biography is available on: <https://www.unibo.it/sitoweb/marco.dallarosa/cv-en>

INNOVATIONS IN FOOD SCIENCE AND TECHNOLOGY AS A SOCIAL AND ENVIRONMENTAL CHALLENGE

Marco Dalla Rosa¹

¹*Alma Mater Studiorum Università di Bologna, Italy*

**Corresponding author: email address of corresponding author*

PL

Food processing to maintain as much as possible sensorial quality as well as texture, aroma, acceptability and functionality is developing toward a less intense treatments than in the past, where candying, strong drying and heat stabilization treatments have been used for centuries. Mild processing concept is evolving trying to keep original-food colours, texture and flavour together with the maintenance of the highest nutritional values. Furthermore, the possible addition of new component able to increase the food functionality is appearing to give much more added value to the new product concept (as well in the case of the minimally processed fruit). On the other hands, new processing techniques based not (only) on heat exchanges can lead to a processing energy demand reduction as well as food losses and waste reduction. The aim of this presentation will be to give an overview on the mild, non thermal and new processing techniques used to minimally process of mildly processing mainly fruit and vegetables but not only, with the purpose of quality and energy saving in respect of the conventional processing methods.

Prof. Luciano Saso

Faculty of Pharmacy and Medicine, Sapienza University of Rome, Italy



Prof. Luciano Saso is a Member of the Faculty of Pharmacy and Medicine, Sapienza University of Rome, Italy (<http://en.uniroma1.it/>). He is author of more than 180 original scientific articles published in peer reviewed international journals with impact factor (H-index Google Scholar= 32, H-index SCOPUS = 26, Total Impact Factor = 479) working mainly in the field of **oxidative stress and antioxidants**. He coordinated several international research projects and has been referee for many national and international funding agencies and international scientific journals in the last 20 years. He has been Guest Editor of the following Special Issues: “**Oxidative Stress as a Pharmacological Target for Medicinal Chemistry: Synthesis and Evaluation of Compounds with Redox Activity**” published in the journal Current Topics in Medicinal Chemistry in 2014, “**Synthesis, evaluation and pharmacological applications of antioxidants**” published in the journal Curr Med Chem in 2013, “**Antioxidant heterocyclic compounds in drug discovery and medicinal chemistry**” published in the journal Mini reviews in medicinal chemistry in 2013, “**Chemistry and biology of antioxidants**” published in The Journal of Pharmacy and Pharmacology in 2007. The special issue **Modulation of Oxidative Stress: Pharmaceutical and Pharmacological Aspects** for the journal Oxidative Medicine and Cellular Longevity is currently in press. Further information can be obtained at www.researchgate.net/profile/Luciano_Saso or contacting luciano.saso@uniroma1.it

MODULATION OF OXIDATIVE STRESS AS A PHARMACOLOGICAL STRATEGY

Luciano Saso

Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy

*Corresponding author: luciano.saso@uniroma1.it; www.researchgate.net/profile/Luciano_Saso

PL

Oxidative stress (OS) is linked with many pathologies ranging from cancer to neurodegenerative diseases and modulators of oxidative stress can be of value in some of these diseases. Different direct and indirect mechanisms by which OS can be modulated will be presented, including scavenging and metal chelating effects, mimicking the antioxidant enzymes or upregulation of their expression, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and inhibition of pro-oxidant enzymes among others. The reasons that may explain why redox therapies failed in many instances will be illustrated. Novel approaches to redox therapies are necessary and the availability of biomarkers capable to predict the relative clinical response is crucial.

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Professor Dr. GIACINTO BAGETTA

Department of Pharmacy, Health Science and Nutrition, University of Calabria, Italy



Professor Bagetta is a full professor at the Department of Pharmacy, Health Science and Nutrition, University of Calabria, Italy. He obtained a degree in Medicine and Surgery (cum laude) in 1983. Prof. Bagetta has been a Consultant in Pharmacology since 1987 and a Full Professor of Pharmacology since 1994. He is author of more than 400 publications: more than 160 full papers (see PubMed) in internationally indexed journals (h-index 38, Citations > 5000; Top Italian Scientist and Top Ten Scientist of the University of Calabria established by Via-Academy), 25 book chapters, and more than 250 abstracts of communications in national and international meetings. Prof. Bagetta is a Member of the Italian Societies of Pharmacology, Neuroscience and a former member of the Italian Association of Immunopharmacology, British Pharmacological Society, and International Society for Neurochemistry. He is also a member of the Editorial Board of high impact factor journals like *Current Opinion in Pharmacology* and a past member of the Editorial Board of *Journal Neurochemistry*, *Journal of Neuroscience Methods*, and *Journal of Chemotherapy*. Prof. Bagetta is a referee for prestigious, internationally recognized scientific journals and research funding agencies such as The Wellcome Trust (UK), The National Council for Research (CNR, Italy), The Italian Space Agency (ASI), and the Ministry of Scientific Research of the Austrian Government; Member of the National Health Research Committee at the Ministry of Health since 1996 and with right of vote from May 2004 to December 2010. Expert member in Pharmacology for ethic committees at the regional hospitals and Italian Institutes for Clinical Research (IRCCS) since 1996 through 2013.

RATIONAL BASIS FOR TRANSLATION OF BERGAMOT ESSENTIAL OIL AS NEUROTHERAPEUTIC

Giacinto Bagetta¹, Tsukasa Sakurada², Maria Tiziana Corasaniti³, Shinobu Sakurada⁴

¹ Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, Rende, Italy;

² Drug Innovation Center, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan;

³ Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy;

⁴ Department of Physiology and Anatomy, Tohoku Pharmaceutical University, Sendai, Italy

*Corresponding author: g_bagetta@virgilio.it

The essential oil (EO) of bergamot is used in aromatherapy to minimize stress-induced anxiety, mood disorders and chronic pain. Preclinical data show that for systemic administration bergamot EO is endowed with behavioral and EEG effects being a functional reflection of the phytocomplex interfering with fundamental synaptic processes (Morrone et al., 2007) and plasticity (Bagetta et al., 2010). Peripheral nerve damage causes neuropathic pain manifested by hyperalgesia and allodynia and bergamot EO has been shown to be effective in controlling neuropathic pain (Katsuyama et al., 2015, Rombolà et al., 2016). In fact, it attenuates mechanical allodynia in the spinal nerve ligation model of neuropathic pain in mice as well as in the partial sciatic nerve injury model. Quite importantly, neuropathic pain is often resistant to opioids and neither systemic nor i.t. morphine is effective. Interestingly, in neuropathic mice, intraplantar injection of very low doses of morphine induces a dose-dependent analgesic effect when combined with inactive doses of bergamot EO or linalool, an ingredient of the essential oil. The latter effect originates from interactions with primary afferent neurons and is important since unwanted side effects in the CNS are avoided. Collectively, the preclinical data gathered so far from the rational basis for translation of EO of bergamot in pain trials.

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Assoc. Prof. Souwalak Phongpaichit

Faculty of Science, Department of Microbiology, Prince of Songkla University, Thailand



Assoc. Prof. Souwalak Phongpaichit is a member of the Faculty of Science, Prince of Songkla University, Thailand (<http://www.sc.psu.ac.th/>). She is author of more than 120 original scientific articles published in peer reviewed international journals with impact factor (H-index Web of Science = 26; H-index SCOPUS = 28, Total Impact Factor = 273) working mainly in the field of antifungal metabolites and fungal diversity. She has been a referee for many national funding agencies and international scientific journals in the last 30 years.

Further information available on:

<http://www.sc.psu.ac.th//New56/EN/StaffDetail.asp>

or via email: souwalak.p@psu.ac.th

BIOACTIVE FUNGAL METABOLITES

Souwalak Phongpaichit¹, Vatcharin Rukachaisirikul², Jariya Sakayaroj³

¹ Natural Product Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand,

² Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand,

³ National Center for Genetic Engineering (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand

*Corresponding author: souwalak.p@psu.ac.th

KEYWORDS: endophytic fungi; sea fan-derived fungi; soil fungi, antimicrobial activity; cytotoxicity

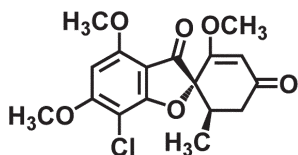
INTRODUCTION: Drug-resistant microorganisms are a major public health concern worldwide. There is an urgent need to find new effective antimicrobial agents from new sources. Fungi have been known to be a good source of bioactive metabolites since the discovery of penicillin. Over the past two decades, investigations of endophytic fungi from various medicinal plants and marine-derived fungi as well as soil fungi have been shown to produce a wide range of bioactive substances including antimicrobial and cytotoxic compounds.

OBJECTIVES: To search for potential fungi from various sources that produce bioactive metabolites against drug-resistant pathogens and selected cancer cell lines.

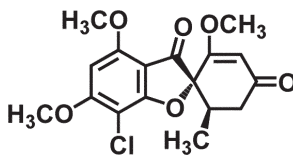
METHOD / DESIGN: Endophytic fungi from various *Garcinia* plants, mangrove plants and seagrasses, seafan-derived fungi and soil fungi were isolated, grown in broth media and extracted with organic solvents. The fungal crude extracts were preliminary screened for their antimicrobial activities against human pathogenic microorganisms including methicillin-resistant *Staphylococcus aureus* (MRSA) by colorimetric broth microdilution tests and against human breast cancer (MCF-7), human oral carcinoma (KB) and noncancerous (Vero) cell lines. Potential fungi were identified based on their morphological characters and molecular techniques based on the ribosomal DNA-internal transcribed spacers (ITS1-5.8S-ITS2, ITS) analyses. Bioactive metabolites were isolated and their structures elucidated using spectroscopic data.

RESULTS: One hundred and forty-two compounds including 49 new compounds were obtained from 17 selected *Garcinia*-associated endophytic fungi along with 83 compounds (28 new) from nine mangrove-derived fungi, 45 compounds (15 new) from ten seagrass-derived fungi, 144 compounds (45 new) from 13 seafan-derived fungi and 97 compounds (29 new) from seven soil fungi. Among them, griseofulvin isolated from the *Garcinia*-derived endophytic fungus PSU-N24 showed the best antifungal activity against a ringworm fungus *Microsporum gypseum* with a minimum inhibitory concentration (MIC) value of 2 µg/mL, whereas emodin obtained from the sea fan-derived fungus *Trichoderma aureoviride* PSU-F95 and also from the seagrass-derived fungus *Bipolaris* sp. PSU-ES64 showed the best inhibitory activity against MRSA with an MIC

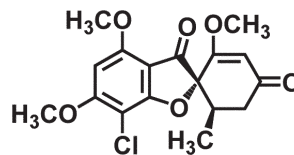
value of 4 $\mu\text{g}/\text{mL}$, and austrocortinin from the sea fan-derived fungi *Fusarium* spp. PSU-F14 and F-135 was cytotoxic on MCF-7 cell lines with an IC_{50} value of 6.3 μM but was non-toxic to Vero cells.



Griseofulvin
Anti-M. gypseum
(MIC 2 $\mu\text{g}/\text{mL}$)



Emodin
Anti-MRSA (MIC 4 $\mu\text{g}/\text{mL}$)



Austrocortinin
Cytotoxicity on
MFC-7 (IC_{50} 6.3 μM)
Vero cells (non-toxic)

CONCLUSIONS: Obtained results show that fungi are a good source of bioactive and new compounds.



T1

ABSTRACTS

Track 1: Biodiversity and Environment





Sara Bumrungsri

ECOSYSTEM SERVICES OF BATS IN THAILAND, A CASE STUDY

T1

Sara Bumrungsri¹, Kitichate Sridith¹, Paul Racey²

¹Department of Biology, Prince of Songkla University, ²University of Exeter, UK

*Corresponding author: sarabumrungsri@gmail.com

KEYWORDS: Bats; *Chaerephon plicatus*; *Eonycteris*; pest control; pollination; rice.

INTRODUCTION: Ecosystem services of bats are vital to our livelihood. Nectar bats in Southeast Asia are key pollinators to economic plants (durian and *Parkia*) while insectivorous bats are key insect control agents in rice fields.

OBJECTIVE: To determine if nectar bats are key pollinators of durian and *Parkia* spp. and to examine how much an insectivorous bat, *Chaerephon plicatus* can save rice production in Thailand.

METHOD: The floral biology and pollination ecology of durian, *Durio zibethinus*, were determined in eight semi-wild trees, while 28 trees of *P. speciosa* and four *P. timoriana* were examined. Diet, population and distribution of *C. plicatus* in Thailand were determined

RESULT: Durian and *Parkia* spp. are mostly or completely self incompatible. Flowers open fully at late afternoon or evening, and anthers dehiscence around 2000 h when the stigmata are already receptive (durian) or receptive at same time (*Parkia*). In a series of pollination experiments, the greatest pollination success occurred either after hand-crossed (durian) or open pollination (*Parkia*). Insect pollination resulted in fruit set in only 12% of *P. speciosa* inflorescences. Fruit bats, mainly *Eonycteris spelaea*, visit flowering plants continuously from dusk till after midnight. Nocturnal and diurnal insects (moths, and giant honey bee, stingless bees) also frequently visit flowers. The pollination services of fruit bats to these plants were estimated to be 137 billion US Dollar in southern Thailand. Since these food plants depend on fruit bats as their pollinators, while *E. spelaea* appear to be declining throughout their distribution, therefore protecting fruit bat populations and their roosts is vital for the production of these fruit crops. The Wrinkle lipped bat, *C. plicatus*, feed largely on planthopper, the main rice pest. Most of the colonies in Thailand are surrounded with rice field. Based on one pest species consumption, this bat was estimated to save 2,900 tons of rice in Thailand.

CONCLUSION: Nectar bats, mainly *E. spelaea*, are principal pollinators for durian and *Parkia* spp. While *C. plicatus* can save rice production in Thailand up to 2,900 tons.



Snežana Radenković

DIVERSITY AND CONSERVATION OF HOVERFLIES (INSECTA: DIPTERA: SYRPHIDAE) IN SOUTHEAST EUROPE

Snežana Radenković¹

¹*Department of Biology and Ecology, Faculty of Sciences, 2 Trg Dositeja Obradovića, 21000 Novi Sad, Serbia*

*Corresponding author: snezana.radenkovic@dbe.uns.ac.rs

KEYWORDS: syrphids, endemics, the Balkans, protection

INTRODUCTION: Hoverflies (Diptera: Syrphidae) are one of the well-known groups of two-winged flies in Europe, with almost 900 species recognized as of now (Speight, 2014). They are an important pollinator group in nature, owing to the diet (pollen and nectar) of adults, which includes a wide spectrum of plant species. The high number of different functional groups and diverse larval ecology (phytophagous, saprophagous, zoophagous, microphagous) make them excellent ecosystem indicators.

In Southeast Europe, different authors have made a great contribution to fauna, zoo-geography, taxonomy, and phylogeny of these insects, especially in the last 65 years. Complex geological history of the area resulted in a very diverse fauna (with more than 450 registered species), rich in endemics and relicts. The high number of hoverfly species from this region has been described during the past decades.

The decline in hoverflies, besides honeybees and wild bees, has been documented in some parts of Europe (STEP, 2016). Different factors like climate change, loss of plants, fragmentation and habitat loss, pests, diseases, and alien invasive species cause this negative trend in pollinators. However, there have been very few conservation studies with focus on hoverflies. These studies are mainly concerned with National Red Lists or the strategy for protection of invertebrates (mostly saproxylic species). The Republic of Serbia is one of the few countries in Europe that has legally protected hoverfly species (Code on declaration and protection of strictly protected and protected wild species of plants, animals and fungi; “Official Gazette of RS”, no. 5/2010 of 5.2.2010) and is the first country to put a site under protection due to diversity and importance of syrphids.

OBJECTIVES: This paper presents an overview of richness of the hoverfly fauna in Southeast Europe, with emphasis on endemic species. The practical measures for improving the protection of this important pollinator group, which are the result of many years of research, are also discussed here.

METHOD: During the last 55 years, adult specimens have been collected throughout the territory by different collectors. Some areas have been explored more intensively due to the presence of diverse habitats or some specific ecosystems. All records are georeferenced and stored in the database of the Faculty of Sciences, University of Novi Sad.

RESULTS: In Southeast Europe over 100 endemic species from 25 genera (*Anasimyia* Schiner, 1864, *Brachyopa* Meigen, 1822, *Chalcosyrphus* Curran, 1925, *Cheilosia* Meigen, 1822, *Chrysogaster* Meigen, 1800, *Chrysotoxum* Meigen, 1803, *Epistrophella* Dusek et Laska, 1967, *Eristalis* Latreille, 1804, *Eumerus* Meigen, 1822, *Katara* Vujić et al., in prep., *Melanogaster* Rondani, 1857, *Merodon* Meigen, 1803, *Myolepta* Newman, 1838, *Orthonevra* Macquart, 1829, *Palumbia* Rondani, 1865, *Paragus* Latreille, 1804, *Pipiza* Fallen, 1810, *Pipizella* Rondani, 1856, *Platycheirus* Lepeletier et Serville, 1828, *Psilota* Meigen, 1822, *Riponnensia* Maibach, Goeldin et Speight, 1994, *Simosyrphus* Bigot, 1882, *Sphegina* Meigen, 1822, *Sphiximorpha* Rondani, 1850 and *Spilomyia* Meigen, 1803) have been registered. Almost half of the endemics belong to large phytophagous genus *Merodon* with the highest diversity in the Mediterranean region. Other two phytophagous genera, *Cheilosia* and *Eumerus* also contain a considerable number of species with restricted range. It is especially important to emphasize the existence of monotypic genus *Katara connexa* (Vujić et al., in prep.), recently described from Pindos mountains in central Greece. This area belongs to the Biome of Oromediterranean mountains, rich in preglacial relicts. From the same locality (*Katara*, 1300 m.a.s.l.), another endemic species *Cheilosia katara* was described (Claussen & Vujić, 1993). Both species are only known from this spot with relict coniferous forests (*Pinus heldreichii* and *P. nigra* ssp. *pallasiana*). Elements of Oriental and Afrotropical fauna also meet in Southeast Europe. *Palumbia erystalodes* (Portschinsky, 1887) is Asiatic (Transcaucasus, Turkey, Lebanon) species registered in Greece. *Simosyrphus scutellaris* (Fabricius, 1805) is Oriental species that reaches the southern Palaearctic, as far west as NE Greece (Speight, 2014). The range of Afrotropical species *Eumerus obliquus* (Fabricius, 1805) extends to southern France, Italy, and islands in the Mediterranean Sea. Defining of Prime Hoverflies Area network - case study in Serbia (Vujić et al., 2016), is suggested as a conservation tool that could also be applied in other countries. The reason is that habitats of various species do not always overlap with protected areas and this gap is especially evident in the less appealing groups of organisms, especially invertebrates. The following steps in designing the network for hoverfly conservation have been suggested: 1) development of species distribution model; 2) fieldwork correction, 3) delimitation of the network based on expert opinion, and 4) evaluation of the network using gap and irreplaceability analyses.

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**Anchana Prathep**

SEAGRASS BED AS A CARBON SINK IN RANONG BIOSPHERE RESERVE AND TRANG-HAAD CHAO MAI MARIN NATIONAL PARK: AN IMPORTANT ROLE OF SEAGRASS**Anchana Prathep¹**, Piyalap Tuntiprapas¹

¹ *Seaweed and Seagrass Research Unit, Excellence Centre for Biodiversity of Peninsular Thailand, Department of Biology, Faculty of Science, Prince of Songkla University, HatYai, Songkkla, Thailand 90112*

*Corresponding author: anchana.p@psu.ac.th

KEYWORDS: Biomass; Blue Carbon; Carbon Storage; Seagrass

Drastic increasing of carbon dioxide in an atmosphere is critical and has been center of various recent research fields. This is a detailed study of how seagrass can help storing the carbon in their ecosystem. The study was carried out in 2 different sites (the Ranong Biosphere Reserve (RBR) and Trang Haad Chao Mai Marine National Park (HCM).) and 2 degrees of seagrass coverage (80% VS 20% coverage), which 80% coverage represents a healthy seagrass bed and 20% coverage represents an unhealthy bed. We examined the % carbon content of a rich diversity of seagrass species, compared the above and below ground plant parts and the carbon content in the sediments according to the grain sizes > or < 63µm. The results showed that there were differences between the above and below ground tissues, between study sites, among species and between coverages ($P<0.05$). The healthy seagrass bed had greater carbon content. The sediment fractions were also different at different study sites and also those associated with different species ($P<0.05$). Most sediments were composed of particles greater than 63µm. The % carbon was greater in the sediment with particle size less than 63µm. The total amount of carbon stored in live seagrasses at RBR is estimated to be 6.08 Mg and at HCM, 335.16 Mg. A larger scale estimate of carbon stored in the seagrass ecosystems in Thailand is needed and could help guide the conservation and management of this important but understudied ecosystem.



Milivoje Krvavac

EXPLOITATION OF THREATENED NON – CITES AND NEW DISCOVERED REPTILE SPECIES

Milivoje Krvavac¹

¹*Institute for Nature Conservation of Serbia, Dr Ivana Ribara 91, 11070 Belgrade, Serbia*

**Corresponding author: milivoje.krvavac@zzps.rs*

KEYWORDS: reptile; new species; illegal; trade; CITES

The concept of species conservation has been complemented by broader approaches to environmental challenges, such as the controversial “ecosystem services” concept. However, in view of the increasing numbers of globally threatened species (IUCN 2015), the protection and sustainable conservation of species demands more attention than ever. The IUCN Red List has assessed 45% of the world’s reptile species of which at least 1390 species are threatened by “biological resource use.” Of these, 355 species are intentionally targeted by collectors, including 194 non-CITES-listed species. The exploitation of such species is highlighted. Illegal trade activities involve species regulated under CITES, but concerns are also raised about the provenance of species that are newly discovered or not CITES-regulated but are nationally protected in their country of origin, and are openly offered for sale in the EU. Case studies are presented from the Asian and African continent.

The primary methodological approach for this review was extensive, global-scale consultation with experts who have relevant, long-term experience addressing reptile trade issues. These experts include: scientists, officers of conservation agencies, and enforcement and customs officials. Contributing authors were requested to provide examples of unsustainable and illegal trade activities regarding the collection of, and international trade in, live reptiles.

The targeted harvesting of internationally highly lucrative species, that inhabit specific ecosystems in only single countries or islands even accelerates over-exploitation and extinction processes, compared to impacts by the destruction of natural habitats. The removal of nationally protected and red-listed reptile species not regulated in the CITES Appendices, cannot be enforced outside the species’ native range, which is a

definite gap in international legislation. For rare species that are only protected on a national level in their range state, no legislation exists on an international level, to adequately protect them from exploitation on the international market, once illegally removed from the wild and smuggled out of their range. This article demonstrates that more than 90% of the world's reptiles are neither regulated by CITES or the European Wildlife Trade regulations. It also substantiates that those species regulated under the current laws, are also illegally traded to supply the international reptile pet market. Europe, as the major consuming region worldwide in the live reptile trade, needs to take responsibility for the conservation of species outside its range.



DETERMINATION OF KEY PHYSICO-CHEMICAL PARAMETERS OF WATER BODIES QUALITY BY FIBER OPTIC SENSORS (FOS)

Boris Obrovski¹, Ivana Mihajlović¹, Jovan Bajić², Branislav Batinić², Miloš Živanov²,
Mirjana Vojinović Miloradov¹

¹ *University Of Novi Sad, Faculty Of Technical Sciences, Department Of Environmental Engineering, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia*

² *University Of Novi Sad, Faculty Of Technical Sciences, Department of Power, Electronic and Telecommunication Engineering, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia*

* *Corresponding author: miloradov@uns.ac.rs*

KEYWORDS: surface water; swimming pools; fiber optic sensors; water monitoring

INTRODUCTION: Wastewater discharged from anthropogenic sources and natural processes disrupt the quality of the water body. Contamination of the environment, especially aquatic medium which is used for human activities require high-quality monitoring program due to the potential negative impact on humans and aquatic life. Great efforts have been made in the development of new devices and equipment to provide quality and more reliable monitoring program. Large numbers of fiber optic sensors (FOS) based on different principles were developed and have been applied for detecting different physico-chemical parameters in aqueous media. FOS is increasingly developing for possibility of receiving real-time, immediate and reliable data about the current state of the water bodies.

OBJECTIVES: The aim of this study is to enable calibration of FOS for measurement of key physico-chemical parameters in river water and water from the swimming pool. The results obtained by applied sensor were compared with the results obtained by standard laboratory methods (UV-Vis) to confirm the effectiveness of the FOS device.

METHOD / DESIGN: Samples for laboratory analysis were collected from Danube surface water and swimming pool in the city of Novi Sad, Serbia. Analyses were carried out in accredited Laboratory for monitoring of landfills, wastewater and air, Department of Environmental Engineering and Occupational Safety and Health in Novi Sad. Concentrations of selected physico-chemical parameters in water samples were analyzed according to the standard EPA and HACH Methods. The concentrations were measured with UV-VIS spectrophotometer (DR 5000, HACH, Germany).

Operating principle of implemented FOS is the absorption of light. When the light passes through a liquid, certain wavelengths will be transmitted while others are absorbed depending on the color of the tested liquid. Fiber optic sensor detects the color intensity of sample and converts RGB color model in HSV color model.

RESULTS: FOS was proved to be applicable for monitoring of orthophosphate, nitrite, sulfate, chlorine and hexavalent chromium in surface water in laboratory controlled conditions, as well as residual and total chlorine in samples from swimming pool.

Relative differences for observed parameters measured by FOS were lower than 10 %, confirming the successful usage of optical sensor in laboratory controlled conditions.

CONCLUSIONS: Applied FOS is multiparameter device which can be optimally used for five parameters in river water and two parameters in waters from swimming pool, with possibility of application to other water bodies, such as lakes, ponds and groundwater. One of the great advantages of the applied sensor could be early and prompt information as well as low price of components for the construction of up to date device. Construction of field device with improved performances will provide higher quality monitoring program and more reliable results which is important in the case of contamination and early responses in order to prevent the contamination.

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BEING SMART IN A MULTI-STRESSED WORLD - PREDICTING THE STRESS IMPACT ON *LEMNA MINOR* L., *CERATOPHYLLUM DEMERSUM* L. AND *MENTHA AQUATICA* L. ALONG THE DANUBE RIVER USING ANN TRAINED ALGORITHMS

Maja Novković^{1,*}, Đorđe Obradović², Dušanka Cvijanović¹, Ivana Krtolica², Aleksandar Kaplar², Milica Živković¹, Ivana Teodorović¹, Mirjana Vojinović-Miloradov², Snežana Radulović¹

¹ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

² University of Novi Sad, Faculty of Technical Sciences

* Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia

* Corresponding author: maja.novkovic@dbe.uns.ac.rs

KEYWORDS: Macrophytes; Danube; fuzzy; artificial neural network; impact

Macrophytes play an inevitable role in the ecological monitoring since changes in the composition of aquatic vegetation are reliable and stable indicators of the quality of water and littoral zone of aquatic ecosystems. The most used methods to indicate this role are Cocktail, TWINSpan method, DCA, CA, CCA, DCCA, PCA and non-metric multidimensional scaling NMDS. Although widely used, they still can not accomplish the task of determining ecological dynamic of riverine biota, not without including fuzzy logic and weighting connections between multi-stress parameters in real time. Recording and assessment of aquatic macrophytes was a request for the Joint Danube Survey 2 (JDS2&JDS3), with a new insight regarding occurrence, abundance and specific distribution of macrophytes, based on methodological adaptations adjusted better to the size of the river and permitted more appropriate statistical interpretation. An ad-hoc data base used in this study was compiled using JDS2 data set (ICPDR). From a practical standpoint, ANNs are parallel processing systems that consist of simple processing units, neurons, and directed, weighted connections between them. Artificial neural networks (ANNs) can be characterized as computational models, inspired by information processing capabilities of biological neural networks. They can be used for modeling complex interactions in natural systems. In this study a feed - forward artificial neural network with backpropagation training algorithm is used to extract relationships between land use and three selected aquatic *Lemna minor* L. and *Ceratophyllum demersum* and semiaquatic *Mentha aquatica* L species. The network consists of three layers: an input layer, a hidden layer, and an output layer. Sigmoid function is used as the activation function. Under the assumption that high dimensionality of input data increases the error of the backpropagation algorithm and that dimension reduction will result in a decreased error, PCA has been used for dimension reduction of input data. PCA has resulted in reduction of the input data columns from 134 to 17. Neural network has been modified to have 17 neurons in the input layer, 5 in the hidden layer and 1 in the output layer. Results before and after PCA have been compared.

MICROBIOLOGICAL WATER QUALITY OF SELECTED SURFACE WATERS IN VOJVODINA

Dragana Čučak¹, Slavica Perić², Ivica Tamaš², Dragan Radnović²

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia,

² University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

* Corresponding author: dragana.cucak@dh.uns.ac.rs

KEYWORDS: microbiological water quality, heterotrophic plate count; fecal enterococci; total coliforms

INTRODUCTION: The freshwater microbial communities are good indicators of overall water quality since, due to a short generation time, they promptly respond to fluctuating environmental conditions. Specifically, heterotrophic plate counts (HPCs) are indicative of general organic load exerted on a water body, while fecal indicators (fecal enterococci and total coliforms) give insights into levels of contamination of fecal origin.

OBJECTIVES: The aim of this work was to determine the microbiological water quality of selected surface waters in Vojvodina, Serbia, namely the Krivaja, Obedska bara and Lake Ludaš.

METHOD / DESIGN: The samples were taken in the summer and autumn of 2015 from several sampling sites on each water body. Microbiological analysis was performed using the standard cultivation methods: heterotrophic plate count (ISO 6222), total coliforms (ISO 9308:2) and fecal enterococci (ISO 7899:2). The results obtained were classified according to the classification systems of Kavka et al. (2006) and Kirschner et al. (2009).

RESULTS: Based on the counts of heterotrophs, the Krivaja samples were typically classified as the class III (critically polluted) according to Kohl (Kohl, 1975), in particular during the autumn season. In addition, the numbers of fecal enterococci and total coliforms determined in the three of four sampling sites showed a marked seasonal variations, pointing to a rather poor sanitary condition of the Krivaja (Tab 1.). However, based on the counts of heterotrophs, the surface waters of Obedska bara and Lake Ludaš were classified as the II class indicating a moderate pollution by easily degradable organic matter. Moreover, no excessive numbers of fecal enterococci and total coliforms were found implying an over-all better microbiological water quality when compared to the Krivaja.

Tab. 1 The average counts of heterotrophs, fecal enterococci and total coliforms at the sampling sites of the Krivaja, Obedska bara and Lake Ludaš, Vojvodina, Serbia.

Sample location	Sampling site	Sampling season	Heterotrophic plate count (CFU/ml)	Fecal enterococci (CFU/100ml)	Total coliforms (MPN/100ml)
Krivaja	1	summer	5100		
	2	summer	690		
	3	summer	14625		35000
	4	summer	23666		
	1	autumn	47750	2200	
	2	autumn	60667		
	3	autumn	67667		
	4	autumn			25000
Obedska bara	1	summer		670	
	2	summer			
	3	summer			
	1	autumn	17300		
	2	autumn			
	3	autumn			
Ludaš Lake	1	summer			25000
	2	summer			
	3	summer			25000
	1	autumn		1500	25000
	2	autumn		400	
	3	autumn			
Legend: Classification of pollution level: blue - class I (low); green - class II (moderate); yellow - class III (critical); orange - class IV (high); red - class V (excessive).					

CONCLUSIONS: Our analysis indicated a fairly good bacteriological water quality of the lakes Ludaš and Obedska bara with mostly moderate levels of pollution by easily biodegradable organic matter. However, an elevated number of heterotrophs, as well as the high numbers of fecal coliforms found in the Krivaja reflect a very poor sanitary status of the river water due to a high anthropogenic impact.

REMOVAL OF SELECTED PESTICIDES BY MAGNETIC AND/OR MACROPOROUS ION EXCHANGE RESINS

Maja Lončarski¹, Jovana Blitvin¹, Aleksandra Tubić¹, Jelena Molnar Jazić¹, Snežana Maletić¹, Srđan Rončević¹, Jasmina Agbaba¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, R. Serbia

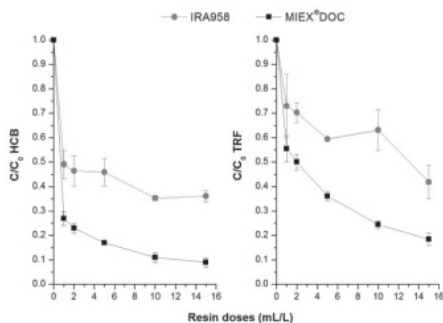
Corresponding author: jovana.blitvin@dh.uns.ac.rs

KEYWORDS: Ion exchange resins; water matrix; hexachlorobenzene; trifluralin

INTRODUCTION: Pesticides hexachlorobenzene (HCB) and trifluralin (TRF) are listed as priority substances by Directive 2013/39/EU, due to their high toxicity and negative impact on the environment. Several available techniques (e.g. advanced oxidation, aerobic degradation, coagulation/flocculation, adsorption, etc.) have been developed for removal of organic pollutants from water. Among the possible alternatives to coagulation/flocculation for removal of organic pollutants, several studies highlighted the strong potential of anion exchange resins (AERs). Various ion exchange resins with a unique continuous ion exchange process (IEP) offer water utilities a cost-effective and environmentally friendly DOC and specific organic pollutants (pesticides, pharmaceuticals, natural hormones etc.) removal process.

OBJECTIVES: The aim of the study was to examine the removal efficiency of two different anion exchange resins (MIEX[®]DOC and IRA958) for selected priority hazardous substances, HCB and TRF from aqueous solution.

METHOD: Laboratory scale experiments were carried out by standard Jar test (FC6S Velp scientific). Synthetic water matrix (500 mL) with initial concentration of 0,72 µg/L for each pesticide (HCB and TRF) were treated by 1,2,5,10,15 mL/L ion exchange resin MIEX[®]DOC (provided by Ixom (Australia)) or Amberlite[™] IRA958 Cl (Rohm and Hass). Contact time was 30 min. with continuous agitation at 150 rpm. Filtered samples (0,6 µm glass fibre filter) were analysed by GC/µECD (EPA 508 method), after liquid-liquid extraction in accordance with modified EPA 3510C method.



RESULTS: Investigated ion exchange resins showed high efficiency removal for hexachlorobenzene and trifluralin (Fig. 1). Results showed that efficiency of IEP increases with increasing resin dose (1-15 mL/L). The percentage of HCB removal was in range from 54% to 73% for IRA958 and from 73% up to 91% for MIEX[®]DOC, depending of applied resin dose. Lower

Fig. 1. IEP efficiency for pesticides removal

removal efficiency was observed during the removal of TRF (28-45% with IRA958 and 45-82% with MIEX[®]DOC). The lower removal efficiency TRF than HCB could be explained by its larger molecule size, which meant it was harder to establish ion exchange on the surface of resins. Comparing removal efficiency of investigated resins, one can observe that MIEX[®]DOC was shown to have better results than the IRA958 resin. This different behaviour can be explained by the higher specific area of MIEX[®]DOC in comparison to IRA958.

CONCLUSIONS: Ion exchange is a relatively simple technique which may be efficiently applied for the removal of hexachlorobenzene and trifluralin from water. MIEX[®]DOC resin effectively removed both HCB and TRF from water, although the IRA958 resin was less effective. Future research should focus on the influence of other water constituents on the performance of these resins, and investigate IRA958 in combination with other processes in order to improve removal efficacy.

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SEAGRASS BIOMASS MAPPING TECHNIQUES IN ANDAMAN COAST OF THAILAND

Milica Stankovic¹, Anchana Prathep¹

¹ *Seaweed and Seagrass Research Unit, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Thailand*

* *Corresponding author: svesemenja@gmail.com*

KEYWORDS: seagrass; biomass; mapping

INTRODUCTION: Understanding monitoring, modeling and managing of the seagrass biodiversity requires spatial information of the meadows. Newer mapping techniques such as remote sensing and drone images integrated with GPS field data are overshadowing the older visual technique due to its potential of the high mapping accuracy. The maps of the seagrass distributions are becoming more common; however mapping of the seagrass biomass has been less frequent. The difficulty of the biomass mapping lies in the requirement of the field collection and the trouble of estimation of the seagrass biomass due to the meadow's patchiness.

OBJECTIVES: The aim of this research was to examine different mapping techniques i.e. regional atlas, visually, satellite and drone images in order to create the most accurate seagrass biomass maps.

METHOD / DESIGN: The study was done in two types of meadow, uniform and mixed in 2015 and 2016 along Andaman coast of Thailand. Biomass samples were collected from high and low density areas from 50×50 cm² plots, with three replications per meadow type. The seagrass meadow distribution maps were obtained from different sources i.e. Asia Air Survey, Japan or from the field visually, with GPS and drone.

RESULTS: The results suggest that biomass maps created from the regional atlases have the lowest quality, while more computerized methods have different rate of success. The level of accuracy varied from very high of drone images, to medium from GPS and visual data and lower accuracy from satellite data. Each of the created maps has its advantages and disadvantages in terms of time, availability and the cost, providing vast possibilities for usage of general public.

CONCLUSIONS: The drone images that provided the highest quality maps could be used for the future studies of monitoring and modeling seagrass meadows, while lower accuracy maps provide enough information for the conservation studies as well as the managing flora and fauna associated with the seagrass meadows.

INFLUENCE OF URBANISATION OF FRUŠKA GORA ON ENVIRONMENT AND BIODIVERSITY

Miljan Šunjević^{1,2}, Mirjana Vojinović-Miloradov², Darko Reba¹,
Faculty of technical sciences

¹ *Department of Architecture and Urbanism,*

² *Department of Environmental Engineering and Occupational Safety and Health*

*Corresponding author: msunjevic@uns.ac.rs

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KEYWORDS: Fruška Gora; environment protection; biodiversity, urbanization

INTRODUCTION: Fruška Gora is first national park that is under protection of state since 1960. Urbanization of Fruška Gora was deep in process when it was pronounced national park and when its protection came into power. But it didn't stopped urbanization process, it only slowed it down. Today we can see its influences and conclude what is current state and what are future steps in protecting its environment and biodiversity.

OBJECTIVES: Objective of research is to determine current state of environment and biodiversity of Fruška Gora and based on that to determine possibility and necessity for biodiversity and environment protection.

METHOD / DESIGN: Determination of current status of biodiversity of Fruška Gora is done by checking previous biodiversity official national reports and biomonitoring of current state. Status of current environment state is determined by checking previously decided environmental markers in present time and through history. Monitoring spread of urban areas on Fruška Gora and observation how it influences changes in biodiversity and environment are the key factors.

RESULTS: Trough history there have been many changes in biodiversity and environment status, but at the moment status has been stabilized and is showing signs of progress. Spread of urban areas on Fruška Gora is more under control and has more respect for biodiversity and environment.

CONCLUSIONS: Although there are signs of progress, there must be more control on urban area spread and its influence on unique environment and biodiversity of Fruška Gora.

TEA WITH GODOT – THE EMERGENCE OF THE PHYLOMORPHOGEOGRAPHY AS A CONCEPTUALLY VALID METHOD FOR THE SUBSPECIES INFERENCE

Marko Djurakic¹

¹University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

*Corresponding author: marko.djurakic@dbe.uns.ac.rs

KEYWORDS: Hermann's tortoise; 2D geometric morphometrics; cytochrome *b*; spatial statistics; integrative taxonomy

INTRODUCTION: From conceptual point of view, the subspecies represent a geographically defined aggregate of local populations which differ morphologically and genetically from other subdivisions of the species. However, the lack of conceptually coherent methods for unambiguous subspecies identification has led to a debate over validity and significance of the subspecies in the estimation of biological diversity and conservation policies.

OBJECTIVES: Here, I present a conceptually consistent method for the subspecies inference which I termed phylomorphogeography and explored its potential to solve subspecific uncertainties in the eastern clade of Hermann's tortoise (*Testudo hermanni boettgeri*).

METHOD / DESIGN: The phylomorphogeography requires georeferenced individual-based morphological and genetic data which are analyzed as independent lines of evidence consistent with the integrative taxonomy standards. First step is the analysis of geographic pattern of genetic variation. Second step is based on a hierarchical clustering of morphological data without *a priori* grouping of individuals. It is crucial that second step is based on an explicit spatial model of the phenotypic data where a number of clusters is estimated with the full Bayesian inference. If genetic and phenotypic data show consistent pattern of geographical clustering of populations, it can be used as a conceptually valid criterion to define the subspecies. To show the utility of the phylomorphogeography I compared the spatial population structure of the eastern clade of Hermann's tortoise (the Balkan Peninsula) using carapace shape and complementary examination of cytochrome *b* mitochondrial DNA (cyt *b* mtDNA).

RESULTS: Using the phylomorphogeography approach I found that the Hermann's tortoise populations in the Balkans are divided into two distinct clusters, thus representing two subspecies. For instance, populations along the Adriatic coast represent a divergent allopatric evolutionary unit compared to the populations from central and southern Balkans.

CONCLUSIONS: The phylomorphogeography represents powerful tool that can bridge the gap between subspecies conceptualization and its operational identification. As the subspecies is the only taxonomic category inferior to species that is recognized

by the International Code of Zoological Nomenclature, the utility of the phylomorphogeography can aid new perspectives to established more accurate science of taxonomy and biodiversity estimation. This is particularly important because researchers, conservationists, law- and policy-makers, as many others rely heavily on taxonomic information to identify, manage, and conserve important units for conservation in the era of biodiversity crisis. The case study of Hermann's tortoise showed how this issue is important because these populations are prone to illegal harvesting, translocations and habitat degradation. Likewise current regulations (e.g. IUCN, CITES) do not recognize subspecies diversity that I observed in this study largely due to lack of the methods to identify such subtle, but valuable intraspecific variation.

FOREST RESERVES IN SERBIA – THE STATUS, DISTRIBUTION AND SYNECOLOGY

Dragana Ostojčić¹, Vladimir Nikolić¹, Aleksandar Dragišić¹

¹ *Institute for Nature Conservation of Serbia, Dr Ivana Ribara 91, 11070 Belgrade, Serbia*

*Corresponding author: dragana.ostojic@zzps.rs

KEYWORDS: forest reserves; conservation; state; distribution; synecology.

Nature conservation in Serbia has begun with the designation of strict forest reserves, as the most conserved parts of nature. The first nature reserve was designated in 1948 under the name “Zeleničje” on the mountain Ostrozub. Since then it has begun the institutional (legislative) nature protection, which was being carried out by the Institute for Nature Conservation of Serbia, an institution engaged in nature conservation for more than 60 years. Nature reserves are the most important sites for the conservation of species and ecosystem diversity in Serbia. Moreover, they are the most important categories for the biodiversity conservation in the international classification system. The objective of this paper is an overview of the strict nature reserves, their state, distribution and synecology. The research results that are part of the Studies on protection were used in writing this paper. Data analyses showed that in the past six decades 123 nature reserves were protected, of which 98 were forest reserves, making up more than 20% of the total number (465) of protected natural sites in Serbia. On the basis of the data processing and the outcome results, it has been defined the classification of nature reserves into the appropriate categories and groups according to the tree species, their communities, the degree of threat and their conservation. It has also been determined that there are pure and mixed reserves in Serbia. Pure reserves are composed from: beech, oak, fir, pine, birch and spruce, while mixed forest reserves include: beech, oaks, fir-beech-spruce, and Pancic’s spruce, in the mixed composition. The most distributed forest reserves are those formed of communities of beech and various species of oak. It was also determined that, according to the state, i.e. the degree of conservation of protected forest ecosystems within the nature reserve, two groups were distinguished: 1. Conserved, pristine forest ecosystems (rainforests and forests in high mountainous areas) and 2. Less conserved forest ecosystems of altered primary composition. According to the degree of threat, the reserves are divided into two groups: 1) Forest ecosystems threatened by biotic factors and 2) Forest ecosystems threatened by abiotic factors. The results show that it is necessary to define and implement additional appropriate measures that would enable further conservation of forest reserves as the primary and most important representatives of biological diversity on the territory of Serbia.

EFFECT OF VARIOUS TEMPERATURES ON *BORDETELLA BRONCHISEPTICA* SIPHOVIRUSES INFECTIVITY

Aleksandra Petrovic¹, Verica Aleksic¹, **Petar Knezevic**¹

¹ Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

*Corresponding author: petar.knezevic@dbe.uns.ac.rs

KEYWORDS: *Bordetella bronchiseptica*; temperature; siphoviruses; viability

INTRODUCTION: *B. BRONCHISEPTICA* is a respiratory tract pathogen of animals and an opportunistic pathogen of immunocompromised humans. Recently, we characterized several temperate *B. bronchiseptica* bacteriophages belonging to *Siphoviridae* family, which are involved in bacterial virulence.

OBJECTIVES: The aim of the study was to examine an effect of temperature on *B. bronchiseptica* phage infectivity (vB_BbrS_CN1, vB_BbrS_CN2, vB_BbrS_LK3, vB_BbrS_MW2 and vB_BbrS_FP1).

METHOD / DESIGN: In brief, after 30 minutes of phage (10^5 PFUml⁻¹) exposure to different temperatures in SM buffer (4 °C, 37 °C, 45 °C, 55 °C, 65 and 75 °C), treated suspensions were mixed with soft top agar containing original host (*B. bronchiseptica* ATCC 10580). The inoculated top agar was poured onto Luria-Bertani agar and after the overnight incubation at 37 °C, plaques were counted. Results were present as percentage of infective phages present in the suspensions after treatment, regarding to control. Since the phages were most stabile at 10 °C, PFU on this temperature was used as a control.

RESULTS: All phages showed relatively good stability at all tested temperatures, with an exception of 75 °C. This temperature completely inactivated all phages during 30 min. The best stability showed phages vB_BbrS_LK3 and vB_BbrS_CN2, with approx. 70% of PFU infectivity retaining at 65 °C, followed by vB_BbrS_CN1 phage with 60% viability retaining at the same temperature. In contrast, phage vB_BbrS_MW2 was less stable, as approx. 50 % of virions were inactivated at 45 °C.

CONCLUSIONS: It is well known that among phages, members of family *Siphoviridae* possess the most stable virions. However, our results indicate that phages vB_BbrS_LK3, vB_BbrS_CN1 and vB_BbrS_CN2 are stable even in the conditions equivalent to pasteurization. The data are of great importance, as these temperate phages are involved in bacterial virulence and pasteurization is not a barrier for their transmission.

INSIGHTS INTO *DIAPORTHE* SPECIES COMPLEX FROM SOYBEAN

Kristina Petrović¹, Luca Riccioni², Miloš Vidić¹, Vuk Đorđević¹, Jegor Miladinović¹

¹ Institute of Field and Vegetable Crops, 21000 Novi Sad, Serbia

² Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria-Centro di ricerca per la patologia vegetale, I-00156 Rome, Italy

*Corresponding author: kristina.petrovic@ifvcns.ns.ac.rs

KEYWORDS: *Diaporthe*; soybean; identification; new species

INTRODUCTION: The genus *Diaporthe* contains large number of plant pathogens and endophytes on a wide range of hosts including economically important crops. Four *Diaporthe* taxa are described as soybean pathogens: *D. sojae*, causal agent of pod and stem blight; *D. caulivora* and *D. aspalathi*, agents of northern and southern stem cankers, respectively; and *D. longicolla*, the most common and most damaging agent of soybean seed decay. Taxonomy of *Diaporthe* species was subject of many studies for a long time. Initial identification was based on morphological and cultural characteristics. Currently, taxonomy of *Diaporthe* species are redefined mostly used a combination of morphological features and DNA sequences.

OBJECTIVES: The objectives of this study were to identify the *Diaporthe* species that are found on soybean seeds in Serbia, and to provide clear morphological profile for each species following by molecular identification and pathogenicity test.

METHOD / DESIGN: Isolation and morphology Using standard phytopathological methods, 160 *Diaporthe* strains were isolated from diseased stem tissues and seeds collected throughout the soybean-producing area in Vojvodina Province, Serbia. Study included four *D. aspalathi* isolates from the USA and three *Diaporthe helianthi* strains isolated from sunflower stem. A total of 167 isolates represented different morphological groups recognised on potato-dextrose agar (PDA).

Sequence Analysis PCR amplification and sequencing of internal transcribed spacer (ITS1-5.8S-ITS2) region of rDNA, partial translation elongation factor 1 alpha (EF-1 alpha) and partial large ribosomal subunit (LSU) was performed. BLAST searches were carried out in order to select closely related sequences from GenBank. Phylogenetic trees were inferred in PAUP* 4.0b10.

Pathogenicity tests Pathogenicity was tested on plants and seeds of soybean cultivar Sava, using mycelia and conidia. Five plants per pot were inoculated at the V2 growth stages by plug method, while seeds were inoculated using a conidial suspension (10⁶ conidia/ml). Twenty-five seeds were placed on wet filter paper in 90 mm Petri dishes and incubated at 24°C in the dark. The test was set up in four replications. All decayed seeds were counted after 7 days.

RESULTS: Identification Isolates were divided into five different morphological groups identified as five taxa: 51 isolates of *D. caulivora*, 63 isolates of *D. longicolla*,

46 isolates of *D. sojae*, four isolates of *D. aspalathi*, and three isolates of *D. helianthi*. BLAST analyses confirmed morphological identification of *D. caulivora* and *D. aspalathi*. From the third morphological group, 58 isolates were identified as *D. longicolla* and five as *D. novem*. The greatest variability was observed in *D. sojae*. Molecular identification showed that only 20 isolates belong to this taxon, while the rest 26 were identified as *D. eres*, *D. foeniculina* and *D. rudis*. Phylogenetic analyses showed clearly grouping of all identified species and defined their synonyms.

Pathogenicity The inoculation of soybean plants at the V2 growth stages by inserting the mycelium plugs in wounds on the stem showed that isolates of *D. longicolla*, *D. novem*, *D. aspalathi*, *D. caulivora* and *D. foeniculina* were highly pathogenic causing wilting of all plants, followed by 80% of wilting plants that were inoculated with *D. sojae* and 40% inoculated with *D. eres*. Dynamics of wilting plants was uneven. *Diaporthe rudis* did not cause wilting of plants. None of the control plants showed any disease symptoms. Inoculation of soybean seeds by immersing them into conidial suspensions showed that *D. longicolla*, *D. novem*, *D. sojae*, *D. aspalathi*, *D. caulivora* and *D. foeniculina* significantly reduced the germination rate of the seeds. These species caused seed decay with 100%, followed by 72% of seeds inoculated with *D. eres*, and 62% with *D. rudis*.

CONCLUSIONS: Accurate identification of pathogens and precise naming are essential for all aspects of phytopathology. The name *Diaporthe phaseolorum* has been conventionally used for *Diaporthe* species from soybean. However, symptomatological, morphological, molecular and pathogenic observations in our study demonstrated that within the genus *Diaporthe* on soybeans in Serbia present the following species: *D. caulivora*, *D. sojae*, *D. longicolla*, *D. novem*, *D. foeniculina*, *D. rudis* and *D. eres* species complex. It found that all studied species can complete life cycle on soybean plants, which indicates that the soybean very suitable host plant for *Diaporthe* species.

PHYTOACCUMULATION OF METALS IN THREE DIFFERENT SPECIES OF ŠUMADIJA REGION

Snežana Branković¹, **Filip Grbović**¹, Gorica Đelić¹, Zoran Simić²,
Marija Marin³ and Snežana Cupara⁴

¹ *Institute of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Republic of Serbia,*

² *Institute of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Republic of Serbia,*

³ *Faculty of Biology, University of Belgrade, Belgrade, Republic of Serbia,*

⁴ *Faculty of medical Sciences, University of Kragujevac, Kragujevac, Republic of Serbia.*

*Corresponding author: Filip Grbović: filipgrb7@gmail.com

KEYWORDS: metals; plants; phytoaccumulation; translocation

INTRODUCTION: Polluted soil is a widespread and serious environmental problem. One of the first attempts for the assessment of environmental pollution coming from exhaust gases of automobiles in traffic by using plants is based on the analyses of different trees, grasses, and different plants that grow near highways and cities. Biological indicators have been used for many years to detect the deposition, accumulation and distribution of heavy metal pollution. Uptake and accumulation of elements in plants may follow different paths (the foliar surface and the root system). During the past few decades there has been an increase in the use of higher plant as biomonitors of heavy metal pollution in the terrestrial environment.

OBJECTIVES: The aim of this study was to determine the concentrations of 8 metals in the soil and the selected plants of Šumadija region. Three different species have been chosen for investigation of a possibility to accumulate and transport metals. Therefore, the plants were selected from the area nearby the highway, at the point of entrance to the city of Kragujevac. The following plants were studied: *Matricaria inodora* L., *Achillea millefolium* L., and *Crepis setosa* Haller fill.

METHOD / DESIGN: The determination of plant material was performed in the laboratory of the Department of Biology and Ecology, Faculty of Science in Kragujevac, with the help of standard keys for determination. The metal concentrations in plant and soil samples were determined by atomic absorption spectrometry (AAS Perkin Elmer 3300), directly from the solution.

RESULTS: The following metals were identified in the soil in the descending order of concentrations: Ca>Mg>Fe>Cr>Mn>Pb>Zn>Cu. Plant material contained the Ca>Mg>Fe>Zn>Cr>Mn>Cu>Pb, also in the descending order.

Soil concentration of Cr was higher than all limiting values (the maximum allowed concentration, limit and remedial value), while soil concentration of Pb was higher than value that is allowed by legislation of Republic of Serbia.

The concentration of the metals in plants was variable, dependent on the plant species and types of metals. Plant concentrations of Cr in all three investigated species were higher than average and toxic values of Cr concentrations encountered in plant material.

Higher values of Zn in the plant material than in the soil were found in the roots, stem and whole plant of *M. inodora* and *C. setosa*. Root and leaf of *A. millefolium* contained higher concentration of Zn than the soil. Species *M. inodora* and *A. millefolium* showed good ability for translocation of Ca, Mg, Fe, Mn, Cu, and Zn starting from the root towards the leaf. The species *C. setosa* was involved in translocation only of Cu. Quantity of Ca, Mg, Fe, Cu and Cr in all three studied species was higher in aerial parts than in underground parts, while quantity of Pb was vice versa. The species *C. setosa* showed good accumulation of a large variety of metals (Ca, Mg, Mn, Cu, Zn, Pb, Cr), while *A. millefolium* had the best accumulation of Fe.

CONCLUSIONS: The obtained results indicate that all three studied species could have application in bio-indicative methods (as bioindicators) and in accumulation of the above mentioned metals. Further research could provide more information on use of these plants in phyto-remediation.

MICROWAVE-ASSISTED SYNTHESIS OF BIOLOGICALLY ACTIVE NAPHTHENIC ACIDS DERIVATIVES

Bojana Vasiljević¹, Ljubica Grbović¹, Ksenija Pavlović¹, Mirjana Popsavin¹, Simonida Đurić², Vesna Kojić³

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21 000 Novi Sad, Serbia

² Department of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, Serbia

³ Oncology Institute of Vojvodina, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia

*Corresponding author: bojana.vasiljevic@dh.uns.ac.rs

KEYWORDS: derivatives of naphthenic acids; microwave-assisted synthesis; microbiological and antiproliferative activity

INTRODUCTION: Within the framework of *green chemistry*, a noticeable results were obtained in the solvent-free synthesis of ester and amide derivatives of naphthenic acids (NAs) under microwave irradiation. Naphthenic acid benzyl and choline esters, anilides and morpholides were synthesised directly from free carboxylic acids in the absence of solvent and catalyst. The synthesized natural products were evaluated for their *in vitro* antiproliferative activity against MCF-7, MDA-MB-231, HT-29, MRC-5 and A549 human tumor cell lines and susceptibility to growth stimulation of *Pseudomonas* sp. strains.

OBJECTIVES: Development of versatile, mild and efficient method for the synthesis of potentially biologically active compounds from natural materials.

METHOD / DESIGN: Growth inhibition was evaluated by *MTT colorimetric test*. Bacterial isolates *Pseudomonas* sp. were grown in liquid King B medium, at 28 °C for 24h in Microbiology Laboratory, Faculty of Agriculture, University of Novi Sad. The incubation of bacterial isolates was performed on a rotary shaker (120 rpm) and the final concentration of 10⁸ CFU mL⁻¹ were obtained after 24h. The selected bacterial isolates were subjected to 450 µL of NAs or NA derivatives after which the incubation was proceeded. Control treatments were the bacterial strains that are used alone. The growth of a bacterial isolates were determined on spectrophotometer at OD₆₀₀ after 24h and 48h. The assay was carried out in triplicates.

RESULTS: It was observed that the complex mixture of carboxylic acids, with great structure variety, reacted efficiently with different amines or alcohols under high-temperature heating in closed-vessel system of microwave reactor. Efficient, uncatalysed derivatisation of NAs were carried out under solvent-free conditions. Synthesised NA derivatives and NAs have shown a notable antiproliferative activity against certain human neoplastic cells, but were completely inactive towards the normal MCF-7 and MRC-5 cell line. The results of microbiology experiments have shown the growth stimulation of the examined strains with increase of the incubation time.

CONCLUSIONS: In conclusion, a simple, quick and versatile method for the preparation of ester and amide derivatives of NAs under microwave irradiation was achieved. Biological activity studies of synthesised NA derivatives and NAs have revealed notable antiproliferative activity of compounds gain from natural materials. The growth stimulation of *Pseudomonas* sp. strains suggests possibilities of higher degradation potential of NA derivatives in contrast to NAs.

EFFECTS OF DERIVATIVES OF NATURAL NAPHTHENIC ACID ON THE GROWTH OF FIVE *PSEUDOMONAS* STRAINS

Ksenija Pavlović¹, Ljubica Grbović¹, **Bojana Vasiljević¹**, Mirjana Popsavin¹, Dragana Stamenov²

¹ Department of Chemistry, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, 21000 Novi Sad,

² Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad

*Corresponding author: ksenija.pavlovic@dh.uns.ac.rs

KEYWORDS: naphthenic acids; naphthenic acids derivatives; microbiological tests

INTRODUCTION: It is known that the pollutants of the environment are the substances that are emitted in sufficient concentrations leading to pollution of the land, water, plants, animals, microorganisms and people. For that purpose it's important to examine the potential of microorganisms for the purpose of biodegradation i.e. using some pollutants as a source of carbon atoms and energy.

OBJECTIVES: In this work we examined the influence of naphthenic acids (NAs) and their derivatives (methyl esters, amides, alcohols, hydroxamic acids) on the growth stimulation of five *Pseudomonas* sp. strains by monitoring the growth of these strains in liquid cultures.

METHOD / DESIGN: Bacterial isolates *Pseudomonas* sp. were grown in liquid King B medium, at 28 °C for 24 h in Microbiology Laboratory, Faculty of Agriculture, University of Novi Sad. The incubation of bacterial isolates was performed on a rotary shaker (120 rpm) and the final concentration of 10⁸ CFU mL⁻¹ were obtained after 24 h. The selected bacterial isolates were subjected to 450 µL of naphthenic acids or naphthenic acids derivatives after which the incubation was proceeded. Control treatments were the bacterial strains that are used alone. The growth of a bacterial isolates were determined on spectrophotometer at OD600 after 24 h and 48 h. The assay was carried out in triplicates.

RESULTS: The results of microbiological experiments indicated higher stimulative effect of NA methyl esters and NA primary amides, or more precisely, they are more used by test microorganisms for strain proliferation in contrast to alcohols and hydroxamic derivatives. The results and the structure will be presented in detail.

CONCLUSIONS: The research has shown that the largest degree of biodegradation have the naphthenic acids with a smaller number of carbon atoms and number of rings, namely that the acyclic naphthenic acids are more available to biodegradation than the cyclic. It has been confirmed that the naphthenic acids and their tested derivatives stimulated the multiplication of *Pseudomonas* sp. strains. For further characterization of biodegradation degree, besides the examination of the change in strength of the test microorganisms it would be necessary to determine the concentration of derivatives of naphthenic acids after different periods of incubation.

IMPACT OF NANOSCALE ZERO VALENT IRON ON THE SOIL MICROBIAL COMMUNITY: THE ROLE OF MORPHOLOGY AND REACTIVITY

Andrea Rónavári¹, Margit Balázs², Péter Tolmács², Csaba Molnár³, István Kiss², Ákos Kukovecz^{1,4}, Zoltán Kónya^{1,5}

¹ University of Szeged, Department of Applied and Environmental Chemistry, Rerrich ter 1, H-6720 Szeged, Hungary

² Bay Zoltán Nonprofit Ltd. for Applied Research, Institute for Biotechnology, Derkovits fasor 2, H-6726 Szeged, Hungary

³ Synthetic and System Biology Unit, Biological Research Centre, H-6726 Szeged, Hungary

⁴ MTA-SZTE "Lendület" Porous Nanocomposites Research Group, Rerrich ter 1, H-6720 Szeged, Hungary

⁵ MTA-SZTE Reaction Kinetics and Surface Chemistry Research Group, Rerrich ter 1, H-6720 Szeged, Hungary

*Corresponding author: konya@chem.u-szeged.hu (Zoltán Kónya)

KEYWORDS: nanoscale zero valent iron (nZVI); reductive dechlorination; bioremediation; quantitative PCR

INTRODUCTION: Nanoscale zero valent iron (nZVI) is one of the most extensively used nanomaterials for the remediation/treatment of contaminated soil and groundwater targeting mainly chlorinated organic contaminants (e.g., PCB, CAHs, pesticides) and inorganic anions (perchlorate) or metals (e.g. Cr (VI), As(III)). In recent years, numerous studies have examined the impacts of nZVI on temperature, pH value, removal of contaminants, while little is known about how nZVI affects on microorganisms in soil and aquatic ecosystems.

OBJECTIVES: In this study, the effect of precursors (ferrous sulfate and ferric chloride) and reducing agents (sodium dithionite and sodium borohydride) on the morphology and the reactivity of nZVIs were investigated. We also studied the impact of differently synthesized nZVIs on microbial communities with particular regards to the inhibition or biostimulation of microorganisms which play an important role in the remediation process.

METHOD / DESIGN: The morphological characteristics of synthesized nZVIs were examined by transmission electron microscopy (TEM). The crystal structures of iron nanoparticles were analyzed by X-ray powder diffraction (XRD). The general reducing ability of nZVIs was determined by measuring the H₂ gas evolved during nanoparticles digestion in a concentrated sulphuric acid solution. Batch microcosm experiments were performed to evaluate the effect of nZVIs in 0.1 g/L on anaerobic microflora under anaerobic conditions. The concentrations of trichloroethene (TCE), cis-dichloroethene (cDCE), vinyl chloride (VC), acetylene, ethylene, ethane and methane were measured by gas chromatography. The viable anaerobic heterotrophic cell number was determined by plate counting in R2A agar. The relative amount of *Dehalococcoides*,

sulfate reducers and methanogens were measured by quantitative PCR. The change of total eubacteria profile was examined denaturing gradient gel electrophoresis.

RESULTS: Spherical nanoparticles with higher Fe⁰ content (>90%) was observed by using sodium borohydride as reducing agent, while application of sodium dithionite as reducing agent resulted nanostructures with lower Fe⁰ content (between 68.7 and 85.5%). We observed that the application of 0.1 g/L nZVIs slowly decreased the relative amount of *Dehalococcoides* population in all experiments independently from precursors and reducing agent, whereas the total amount of microbes increased. The relative amount of sulfate reducers was higher in the presence of nZVIs reduced by sodium dithionite while relative amount of methanogens decreased. Contrarily borohydride reduced nZVIs dramatically stimulated methanogenesis. nZVIs in this concentration inhibited biological dechlorination in all microcosms independently from applied precursor.

CONCLUSIONS: The findings confirmed that the used iron precursor and reducing agent impose significant influence on the nature of the particles formed. While in case of using sodium borohydride nanoparticles with high reactivity were formed, application of sodium dithionite nanowires with lower reactivity also could be observed. Furthermore, this study showed that iron nanoparticles depending on their characteristics have also diverse action on groundwater microflora which is responsible for the reductive transformation of chlorinated hydrocarbons. The results suggest that it is necessary to select the appropriate type of nZVI in field tests in order to enhance the remediation process.

ONLINE ZOOLOGICAL COLLECTION OF PSU NATURAL HISTORY MUSEUM, THAILAND

Thidawan Saelao¹, Chutamas Satasook¹

¹ *Princess Maha Chakri Sirindhorn Natural History Museum, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand*

*Corresponding author: thidawan.s@psu.ac.th

T1

KEYWORDS: PSU Natural History Museum; collection management; Biodiversity information

INTRODUCTION: Collection in Natural History Museum is necessary for biodiversity study. It contains useful information, which can be used for further conservation, environmental management, and policy planning programmes. The information includes details on species distribution, morphological characteristics and molecular pattern (if present) etc. In the past, getting access to these information is nearly impossible. However, with the development and fast growing of Information and Communication Technology, the gap on information sharing becomes smaller. This online communication is now applicable to biodiversity information and data can be shared worldwide.

OBJECTIVES:

- 1) To create an online system for curator and administrator.
- 2) To globally share biodiversity information with public and researchers.

METHOD / DESIGN:

This project aims at creating a computing application, which can be stored, retrieved, queried, displayed and shared biodiversity information to public. It is designed as a web-based application, which is physically designed in term of the 3-Tier architecture (Figure 1).

- The client tier interacts with the users via their devices, which host a web browser. This application has been designed to serve 3 different groups of users depending upon their roles and needs i.e. general user, curator and museum administrator.
- The application tier hosts all application components. It handles the interface between the web browser client and the data tier.
- The data tier is a database server, which hosts both data sets and database management system.

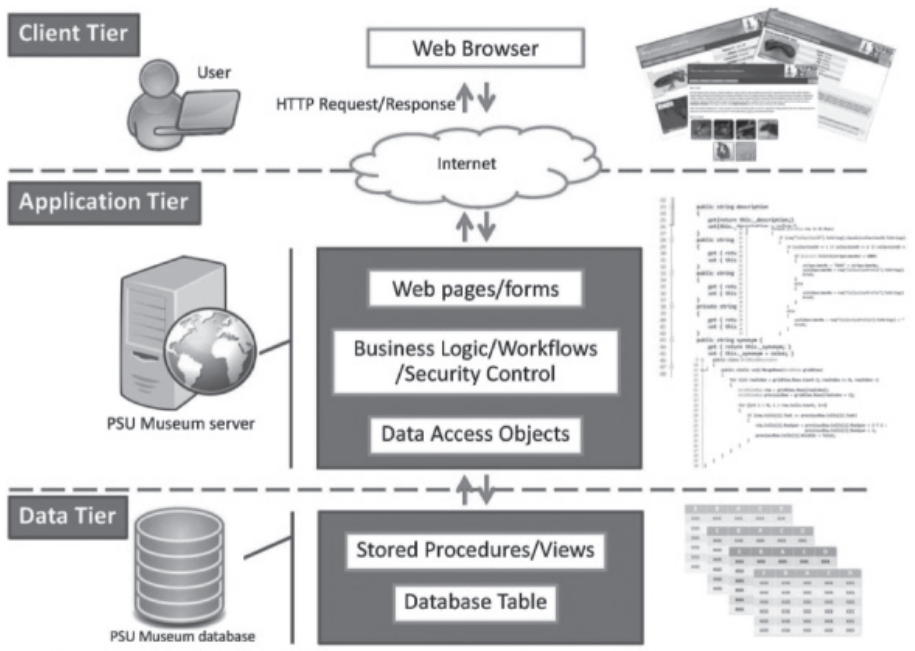


Figure 1 A 3-Tier architecture design for the museum online collection

RESULTS: The Online Collection contains data for each individual specimen that is deposited at Princess Maha Chakri Sirindhorn Natural History Museum (PSUNHM). It provides taxonomic information, identification number, collector, specimen type, locality, sex of specimen, images of specimen and etc. There are now 4 collections completed (Amphibian, Mammal, Reptile, Rodent); and shall be available more of other groups.

It is accessible online at <http://www.nhm.psu.ac.th/collection>. It is also available via Global Biodiversity Information Facility (GBIF) as an occurrence dataset, which can be integrated with other occurrence datasets that contributed to GBIF.

CONCLUSIONS: This online system is a powerful tool for researchers to get access to the biodiversity information of Southern Peninsular Thailand and SE Asian region. This online collection will be extended to cover more organisms and the system will be continuously developed to make it more efficient.

THE ROLE OF PHYSICO-CHEMICAL PARAMETERS IN STRUCTURING MACROPHYTE VEGETATION IN GRAVEL PITS ALONG THE DRINA RIVER FLOODPLAIN (SERBIA)

Bojan Damnjanović^{1,2}, Milica Živković¹, Maja Novković¹, Ana Anđelković³, Snežana Radulović¹, Dušanka Cvijanović¹,

¹ *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia,*

² *Higher Medical and Business-Technological School of Applied Studies Šabac, Hajduk Veljkova 10, 15000 Šabac, Serbia,*

³ *Institute for Plant Protection and Environment, Teodora Dražera 9, 11040 Belgrade, Serbia*

*Corresponding author: bdamnjanovic@live.com

KEYWORDS: Aquatic macrophyte; Physico-chemical parameters; The Drina River; Gravel pits; Canonical Correspondence Analysis (CCA)

INTRODUCTION: In the predominantly agricultural landscape of North-West Serbia, artificial lakes greatly outnumber natural lakes and so make an important contribution to the potential resource for aquatic conservation. The maintenance of healthy aquatic ecosystem is dependent on the physico-chemical properties and biological diversity.

OBJECTIVES: The aim of this study was to estimate an importance of physico-chemical parameters in structuring macrophyte vegetation in gravel pits along the Drina river floodplain.

METHOD / DESIGN: This research was carried out on the six gravel pits in the lower course of the Drina River using the LEAFPACS method during the summer months in 2015. Sampling areas were set along 100 m length sectors. At each macrophyte survey sector, the following set of basic water quality parameters were measured: temperature, dissolved oxygen content, oxygen saturation, electroconductivity, pH, biological and chemical oxygen demand, total organic carbon, total suspended solids, surfactants, nitrates and alkalinity. Canonical Correspondence Analysis (CCA) was performed with vegetation data sets and physico-chemical variables.

RESULTS: Free-floating or rooted submerged pondweed vegetation and rooted waterlily vegetation with floating leaves were recorded in the area studied. All gravel pits could be classified as shallow, mesotrophic, high alkalinity lakes. According to CCA analysis, 24% of the total variance of macrophyte vegetation structure could be explained by physico-chemical parameters. The most significant variables were alkalinity, surfactants, temperature, pH, and total organic carbon (Figure 1).

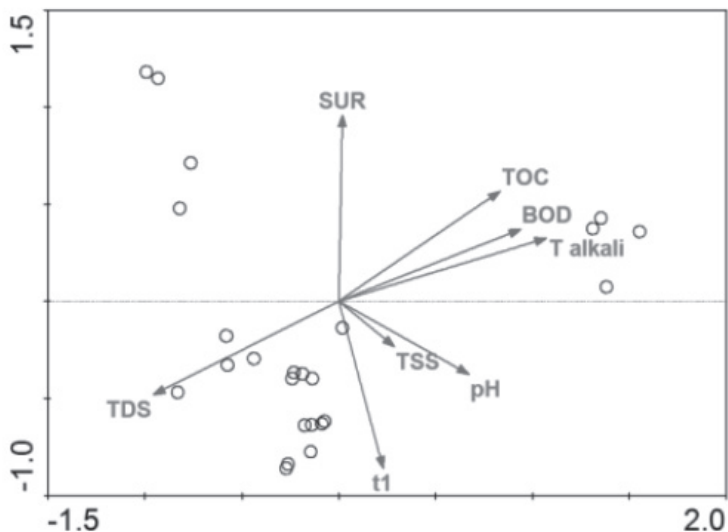


Figure 1. CCA-ordination of all sites with significant physico-chemical variables (First and second CCA axis)

CONCLUSIONS: The present study highlights an importance of land-cover and geology in upstream catchment for structuring macrophyte composition. Upstream catchment geology may influence general physico-chemical parameters such as pH and alkalinity, while the presence of agricultural landscapes may accelerate the eutrophication process. From the perspectives of macrophyte vegetation structure and diversity, as well as water quality, the gravel pits can be seen to have a high conservation value.

THE INTERDEPENDENCY OF HYDROMORPHOLOGY, WATER QUALITY AND MACROPHYTE VEGETATION IN ESTABLISHING CONSERVATION TARGETS FOR EUTROPHIC TEMPERATE LAKES (THE MIDDLE DANUBE BASIN, SERBIA)

Bojan Damnjanović^{1,2}, Maja Novković¹, Milica Živković¹, Ana Anđelković³, Danijela M. Pavlović³, Snežana Radulović¹, Dušanka Cvijanović¹

¹ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia,

² Higher Medical and Business-Technological School of Applied Studies Šabac, Hajduk Veljkova 10, 15000 Šabac, Serbia,

³ Institute for Plant Protection and Environment, Teodora Dražera 9, 11040 Belgrade, Serbia

*Corresponding author: bdamnjanovic@live.com

KEYWORDS: lake; Lake Habitat Survey (LHS); vegetation; macrophyte; conservation; restoration

INTRODUCTION: Major challenges in lake pre-restoration monitoring arise in analysis of the mutual influences of water quality and hydromorphology on aquatic biota. The EC Water Framework Directive (WFD) promotes and encourages the use of standard methods for ecological status assessment. Lake Habitat Survey (LHS) is a widely used method in Europe for determining the hydromorphological condition of lakes.

OBJECTIVES: The aim of this study was to test the usability of the LHS metrics for predicting macrophyte vegetation structure in 31 lakes in the Middle Danube Basin (Serbia), taking into account a covariance with water quality using the partial canonical correspondence analysis (CCA).

METHOD / DESIGN: Field survey was undertaken during the summer months of 2008, 2009, 2010 and 2011 at 31 lakes in Serbia (the Middle Danube Basin).

RESULTS: After accounting for the effects of physico-chemical parameters (28%), hydromorphological variables explained around 17 % of the total variance in macrophyte composition. The partial CCA analysis highlighted the importance of the extent of natural land-cover types in the riparian zone, diversity of natural bank materials, extent of littoral habitat features, hydrological and sediment regime, shore zone modification and intensive use for macrophyte assemblages. Correlation analysis of LHS variables with physico-chemical parameters and macrophyte metrics revealed detailed insights into the relationship between lake ecology and hydromorphology.

CONCLUSIONS: This study demonstrates the utility of the LHS tool for pre-restoration impact assessment and explains the interdependency of hydromorphology, water quality and macrophyte vegetation, providing the important guidelines and a framework for restoration and conservation management of lakes in the area studied.

GROWTH RESPONSE OF OAKS, EUROPEAN BEECH AND SCOTS PINE TO STANDARDIZED PRECIPITATION INDEX

Saša Orlović¹, Dejan Stojanović¹, Bratislav Matović¹, Tom Levanič²

¹ Institute of Lowland Forestry and Environment, University of Novi Sad, Antona Čehova 13d, 21000 Novi Sad, Serbia, sasao@uns.ac.rs, +381 21 540 383

² Slovenian Forestry Institute, Večna pot 2, Ljubljana, Slovenia

*Corresponding author: sasao@uns.ac.rs

KEYWORDS: climate; forest ecosystems; drought; Standardized Precipitation Index (SPI).

INTRODUCTION: Change of climate conditions may lead to various consequences to forest ecosystems in future. It is expected alteration of frequency and character of drought, forest fires, insect and pathogen outbreaks, as well as windstorm events. Changes will be much slower in mounting areas than in lowlands within the same region. Expected range decrease of woody species of 10 m in altitude is predicted to be followed by 1000 times wider range in latitude. From the perspective of the bioclimatic zones, climate change may have opposite effects. In Europe, impact to forestry goes from the expected predominantly adverse effects in Mediterranean, to the generally positive effect in northern boreal regions driven by potential increased precipitation.

METHODS: Standardized Precipitation Index (SPI) was calculating for 3 to 36 months period. Simple Person's correlation was calculated for seven tree-ring chronologies, for four tree species at four different localities. Correlations were ranked to find out which SPI indices provide the best explanation for the growth.

RESULTS: Pedunculate oak stands showed general decline of growth in last 30-40 years, Turkey oak and Scots pine as well, while European beech used to have better growing pattern except for extremely dry period between 2010 and 2013. Generally, growth was positively correlated with SPI at observed year, while it was negatively correlated for the weather conditions of pervious year. SPI 11, 12, 13 and 14 for June, July, August and September express the highest positive correlations with growth for all four species.

CONCLUSION: Species at lower, drier sites (oaks) were more sensitive to long-term effects of precipitation (SPI longer than 12 months), in comparison to species at higher elevation with lower temperature and larger amount of precipitation (beech and pine). Standardized Precipitation Index (SPI) can be an effective tool for assessing climate growth relationship in forestry.

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GROUNDWATER REMEDIATION USING ENVIRONMENTALLY BENIGN ZERO VALENT IRON NANOPARTICLES

G. Kozma^{1,2}, A. Rónavári¹, Á. Kukovecz^{1,2}, Z. Kónya^{1,3}

¹University of Szeged, Department of Applied and Environmental Chemistry, Szeged

²MTA-SZTE "Lendület" Porous Nanocomposites Research Group, Szeged

³MTA-SZTE Reaction Kinetics and Surface Chemistry Research Group, Szeged

*Corresponding author: kozmag@chem.u-szeged.hu

T1

KEYWORDS: nZVI; VOCl, remediation.

INTRODUCTION: Environmental remediation deals with the removal of pollution or contaminants from environmental media such as soil, groundwater, sediment, or surface water for the general protection of human health and the environment or from a brownfield site intended for redevelopment. Nanoscale zero-valent iron particles (nZVI), with sizes smaller than 100 nm, are promising for environmental remediation of polluted water, soil and sediments. Due to its suitable redox potentials, it has been applied to the removal of a wide variety of pollutants including chlorinated hydrocarbons, nitrobenzenes, chlorinated phenols, polychlorinated biphenyls (PCBs), heavy metals, and anions. In this work we compared the performance of sodium borohydride and sodium dithionite derived semi-green nZVI with that of nanoparticles obtained by totally green methods using green tea, coffee and Virginia Creeper (VC) extracts.

OBJECTIVES: Zero-valent iron nanoparticles prepared by using conventional chemicals and natural product extracts are compared and their volatile chlorinated organic degradation efficiency is tested on groundwater samples obtained from an actual nano-remediation site.

METHOD / DESIGN: Green synthesis made from tea, coffee and VC. The extract was prepared by boiling tea, VC leaves or ground coffee in 100 mL deionized water; thereafter the extracts were vacuum-filtered and stored in fridge for further use. Semi-green synthesis methods utilized technical grade ferric chloride, ferrous chloride, ferrous sulfate, NaOH, sodium borohydride and sodium dithionite. For laboratory tests and microscopic (TEM) analysis the as-prepared nZVI particles were collected by vacuum filtration, than rinsed three times with deionized water and ethanol. The samples were kept in stray ambient light at room temperature without stirring to simulate field conditions. Samples were analyzed by TEM, ORP measurement and gas chromatography.

RESULTS: We found that the average particle diameter was between 125-20 nm depending on the synthesis method of the particles. VOCl(27700 µg/dm³) deterioration activity of the semi-green sample series mixed in equimolar amount, x2 and x3 excess to groundwater samples taken from the contaminated well. The concentration of 5000 mg/dm³ has the best efficiency. Higher iron concentration the probability of particle agglomeration increases while lower concentration less efficiency was detected.

Semi-green sample (prepared from FeSO_4 and $\text{Na}_2\text{S}_2\text{O}_4$) was chosen for a scaled-up field test. After two treatments the PCE and TCE were eliminated completely and the amount of VC and DCE was reduced below $15 \mu\text{g}/\text{dm}^{-3}$. In case of green preparation VC reduced nZVI has better properties than coffee, and almost as good as green tea based on ORP and TEM results.

CONCLUSIONS: We compared the reductive dehalogenation efficiency of nZVI particles in removing volatile chlorinated organic compounds (VOCl_s) from groundwater samples obtained from a real remediation target site. Although semi-green methods yielded nZVI particles with smaller diameter and better reducing abilities than green ones. We found that the sustainable optimum nZVI material identified by this study was prepared from FeSO_4 and $\text{Na}_2\text{S}_2\text{O}_4$.

**DISTRIBUTION OF SPECIES OF GENUS HYPERICUM L. 1753
(HYPERICACEAE) IN VOJVODINA (SERBIA)**

Nebojša Kladar¹, Neda Gavarić¹, Milica Rat², Đurđica Simin², Jelena Dugonjić¹, Goran Anačkov², **Biljana Božin**¹

¹ University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia

² University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, Novi Sad, Serbia

*Corresponding author: biljana.bozin@mf.uns.ac.rs

T1

KEYWORDS: *Hypericum*; biodiversity; ecological characteristics; Vojvodina; medicinal plant

INTRODUCTION: St. John's wort (*Hypericum perforatum*, Hypericaceae) has a long history of use in traditional and conventional medicine. It is being used in a form of oil macerates, externally for treatment of burns, bruises, eczema and other skin changes, as well as internally, in therapy of different gastric disorders. Also, of importance is clinically proved antidepressant activity of *H. perforatum* water-alcoholic extracts. Considering the overall broadness of potential therapy indications for *Hyperici herba*, a constant raise of demand for herbal drug on the world market is present.

OBJECTIVES: The objectives of this study were to obtain the data concerning distribution of *Hypericum* species in Vojvodina, Serbia, as well as to analyze basic ecological characteristics of the genus.

METHOD / DESIGN: Distribution data are collected from available literature and from Herbarium of Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad (BUNS). The localities are grouped by regions, and habitats of specific taxa are shown at UTM (Universal Transverse Mercator) map of Vojvodina. The analysis of basic ecological characteristics of the genus included the examination of habitat type, indicator values for basic ecological factors expressed through ecological indexes and life-forms.

RESULTS: Total of seven species of genus *Hypericum* are recorded at territory of Vojvodina with 10 taxa: *H. tetrapterum*, *H. elegans*, *H. hirsutum*, *H. maculatum*, *H. perforatum* (subsp. *perforatum*, subsp. *angustifolium*, subsp. *veronense*, subsp. *latifolium*), *H. humifusum* and *H. x desetangsii*. The results of ecological analyses suggest that recorded plant species are hemicryptophytes which in most of the cases prefer xerophilic open habitats characterized with mildly to moderately fertile soil. The exceptions are *H. tetrapterum* and *H. humifusum* which require wetter habitats.

CONCLUSIONS: The most widespread species of the genus *Hypericum* in Vojvodina is *H. perforatum*, which is of particular importance for pharmaceutical and medicinal use.

ACKNOWLEDGEMENT: Project of Programme of scientific and technological cooperation between Republic of Serbia and Republic of Croatia „New aspects of consumers attitude, safety and quality control of herbal dietary supplements in Serbia and Croatia“ supported this research work.

THE EFFECT OF ACTIVATED CARBON AS A CONTAMINATED SEDIMENT AMENDMENT ON THE BIOAVAILABILITY OF PENTACHLOROBENZENE AND HEXACHLOROBENZENE

Marko Grgić¹, Snežana Maletić¹, Srđan Rončević¹, Jelena Spasojević¹,
Jasmina Agbaba¹, Aleksandra Tubić¹, Jelena Molnar Jazić¹,
Marijana Kragulj Isakovski¹, Božo Dalmacija¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Republic of Serbia

Corresponding author: marko.grgic@dh.uns.ac.rs

KEYWORDS: sediment; pesticides; bioavailability; remediation; active carbon

INTRODUCTION: Interest in the environmental fate of pesticides is motivated by their ubiquitous distribution, their low bioavailability and high persistence in sediment, and their potentially deleterious effect on human health and the environment in general. The use of carbon-rich charcoal-like materials such as activated carbon (AC) for the in situ stabilization of organic contaminants in sediments and soils has thus received increasing attention in recent years. The addition of these materials to soils and sediments has been shown to immobilize organic contaminants thereby reducing their bioavailability to plants, invertebrates and fish. Recently, Europe and the United States have implemented pilot testing of the in situ use of AC as a sediment amendment. Many studies have shown that as a result of the high AC sorption capacity, hydrophobic organic contaminant (HOC) concentrations in porewater and the bioaccumulation of HOCs in benthic organisms are decreased.

OBJECTIVES: The aim of this study was to determine the effect of the AC sediment amendment and the impact of aging sediment amended with AC on the bioavailability of pentachlorobenzene (PCB) and hexachlorbenzene (HCB).

METHOD / DESIGN: In order to assess the impact of the AC amendment and sediment aging with sorption agents on the bioavailability of PCB and HCB, sediment was amended with AC in the following amounts: 0.50%, 1.0%, 5.0% and 10%. Amended sediment was left to age for 14 days and 30 days. After this period, the bioavailability of the PCB and HCB was determined by desorption experiments with XAD-4 macroporous resin. PCB and HCB bioavailability was also assessed in the sediment at zero state, without AC addition. PCB and HCB concentrations were determined on an Agilent 7890A gas chromatograph with a 5975C MSD mass spectrometer on an HP-5MS column (J&W Scientific).

Table 1. Desorption of the PCB and HCB from sediments

Compound	Desorbed amount	Without AC	AC 14 days				AC 30 days			
			0.5	1.0	5.0	10	0.5	1.00	5.00	10.0
C _{pentaclorobenzene}	µg/kg desorbed	3330	237	132	2.43	14.1	38.0	1.45	121	41.0
	% desorbed	99.7	7.11	3.95	0.07	0.42	1.14	0.04	3.62	1.23
C _{hexaclorbenzene}	µg/kg desorbed	1420	388	203	7.60	11.3	124	21.2	111	20.9
	% desorbed	96.6	27.3	14.3	0.53	0.80	8.70	1.49	7.77	1.47

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Table 2. Parameters for the two-fraction model

Pentaclorobenzene	0 days	AC 14 days				AC 30 days			
		0.5	1.0	5.0	10	0.5	1.0	5.0	10
F _{slow} (%)	61.8	92.9	96.3	99.9	99.6	98.8	99.9	96.3	98.7
F _{fast} (%)	38.2	6.55	3.66	0.08	0.43	1.27	0.005	4.21	1.34
Hexaclorbenzene	0 days	AC 14 days				AC 30 days			
		0.5	1.0	5.0	10	0.5	1.0	5.0	10
F _{slow} (%)	30,0	74.5	92.2	99.7	99.7	91,4	98.5	92,3	98.6
F _{fast} (%)	67,1	25.7	7.78	0.34	0.34	9,09	1.67	8,70	1.6

RESULTS: The concentrations of PCB and HCB at the beginning of the experiment were 3340 µg/kg and 1470 µg/kg, respectively. From the desorption results (table 1) it is evident that for both pesticides, the concentrations which can be desorbed from the sediment after a certain aging period are significantly reduced by sediment amendment with AC. Mathematical modeling of the desorption kinetics results, using the nonlinear binomial model according to Cornelissen et al. (1998), allowed the rapidly desorbing fraction to be determined. This fraction is generally considered to be the bioavailable fraction (table 2). Prior to AC addition, 38.2% of the total PCB and 67.1% of the HCB were in the fast-desorbing domain of organic matter. After sediment amendment with AC, the fractions which remain in the fast-desorbing domain of organic matter were reduced to less than 5% for PCB and less than 10% for HCB.

CONCLUSIONS: This study compared the direct connection between the amounts of added AC with the amount of bioavailable chlorinated benzenes in sediment. From the obtained results it can be concluded that activated carbon can be an effective agent for amending sediments contaminated with pentachlorobenzene and hexachlorbenzene, in order to reduce their mobility and toxicity.

Acknowledgments: The authors gratefully acknowledge the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No, III43005 and TR37004).

EFFECT OF UV/H₂O₂ PROCESSES ON THE DEGRADATION AND TOXICITY OF 1,2,3-TRICHLOROBENZENE AND ITS OXIDATION BY-PRODUCTS IN WATER

Tajana Đurkić¹, Jelena Molnar Jazić¹, Aleksandra Kulić¹, Marijana Kragulj Isakovski¹, Aleksandra Tubić¹, Snežana Maletić¹, Jasmina Agbaba¹, Božo Dalmacija¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Republic of Serbia

*Corresponding author: tajana.djurkic@dh.uns.ac.rs

KEYWORDS: 1,2,3-trichlorobenzene, UV photolysis, UV/H₂O₂ process, adsorption, toxicity assessment

INTRODUCTION: The ubiquity of organic micropollutants in waters has raised concerns because of their ability to potentially cause adverse effects even at low concentrations on the environment, aquatic organisms and human health. Among them, chlorinated organic chemicals, including chlorinated benzenes, are defined as priority substances by Directive 2013/39/EU include. These substances are very stable in the environment, toxic and resistant to degradation. In order to improve water treatment, advanced oxidation processes (AOPs) are commonly applied, as they are very effective at treating and degrading various pollutants in different water samples.

OBJECTIVES: The main objective of this research was to investigate the efficiency of 1,2,3-trichlorobenzene (TCB) degradation using direct photolysis and a UV/H₂O₂ advanced oxidation process in natural surface water. The toxicity of the oxidative degradation products was estimated using the *Vibrio fischeri* bioassay.

METHOD / DESIGN: UV photolysis and the UV/H₂O₂ process (1 and 10 mg H₂O₂/L; 100-4200 mJ/cm²) were carried out in a photochemical reactor equipped with a 253.7 nm UV low pressure mercury lamp (Philips TUV 16W). Natural surface water (Danube river) was spiked with an aqueous TCB solution in order to obtain an initial concentration of about 10 µg/L. GC/µECD (Agilent Technologies 6890) was applied for the TCB analysis. The toxicity of water containing TCB before and after UV/H₂O₂ treatment was evaluated using *Vibrio fischeri* (Luminescent bacteria test) using a LUMISTox 300 (Dr. Lange GmbH, Germany).

RESULTS: The results showed that under direct UV photolysis, 2-48% degradation of TCB in natural water was achieved. The UV/H₂O₂ process with a low oxidant dose of 1 mg H₂O₂/L slightly improves TCB degradation (3-69%) compared to photolysis alone. A higher H₂O₂ dose of 10 mg/l was found to be very effective for TCB degradation (28-98%). The greater TCB degradation achieved by the UV/H₂O₂ process at a higher oxidant dose is likely explained by attack of highly reactive and unselective OH radicals. In all investigated processes, the efficacy of TCB oxidative degradation increased with increasing UV dose, reaching a maximum at the highest UV fluence.

During the tests with *V. Fischeri* a very slight bioluminescence inhibition was recorded for untreated samples which was not significantly changed during UV photolysis alone or by the UV/H₂O₂ process with lower dose of peroxide (up to 19% of inhibition). However, applying UV/H₂O₂ treatment with a higher H₂O₂ dose rapidly increased toxicity, reaching a maximum of 78% inhibition, although further treatment with increased UV fluence can reduce this to about 50%. These results are probably a consequence of the formation of degradation by-products of TCB and other organic matter present in natural water and their further degradation with increasing UV dose. Adsorption on powdered activated carbon (10 mg/L) applied after UV/H₂O₂ treatment resulted in additional removal of residual TCB residual (<10 ng/L) and also removed oxidative degradation products, as shown by a significant reduction in bioluminescence inhibition (<10%).

CONCLUSIONS: The UV/H₂O₂ process proved to be very effective for the removal of 1,2,3-trichlorobenzene from surface water. Application of a higher H₂O₂ dose resulted in almost complete TCB degradation (up to 98%), although these reaction conditions led to the formation of more toxic degradation products according to the *Vibrio fischeri* bioassay. Subsequent adsorption on powdered activated carbon was shown to be a good solution for the removal of residual TCB and degradation products from water.

Acknowledgment: The authors gratefully acknowledge the support of the Provincial Secretariat for Higher Education and Scientific Research, Republic of Serbia, Autonomous Province of Vojvodina (Project No. 114-451-2263/2016).

DETERMINATION AND ANALYSIS OF HEAT WAVES FOR THE PERIOD 1961-2000

Tatjana Radišić¹, Nina Novaković²

¹ University of Novi Sad, Faculty of Sciences, Department of Physics,

² University of Novi Sad, Faculty of Sciences, Department of Physics

*Corresponding author: tanjita91@gmail.com

KEYWORDS: Heat wave; Climate change; Frequencies; Intensity

INTRODUCTION: The topic of this work is very actual because it deals with the consequences of global warming. In the last ten years heat waves are more present in all world. In this study we will determine the number of days with heat waves, their intensity and frequencies for one city (Novi Sad) in Serbia.

OBJECTIVES: Our main goal was to find and analyze heat waves for Novi Sad for period 1961-2000. There are many definitions of heat waves but there are no unique. To analyze heat wave duration and frequency, we used heat wave duration index which is recommended by World Meteorological Organization and three different methods.

METHOD / DESIGN: Heat wave duration index-*HWDI* recommended by the WMO is defined as period when the daily maximum temperature more than five consecutive days exceeds the average daily maximum temperature by 5°C, the normal period being 1961– 1990. Since the definition of heat wave duration index is based on the heat wave duration rather than its intensity, to evaluate intensity of heat wave, the peak temperature and cumulative excess was also determined.

Second method was defined heat waves as being three or more consecutive days when daily maximum temperatures reached or exceeded the 95th percentile of the maximum temperature range for the study period. Seasons were defined as: winter-DJF (from December to February), spring-MAM (from Mart do May), summer-JJA (from Jun to August) and autumn-SON (from Semptember to November). The third and fourth method are similar because we used apparent temperature to calculate heat waves. The third method defined heat waves as periods of at least three consecutive days with daily mean air temperature above the 97.5th percentile of the all-season temperature distribution. Fourth methods defined heat waves as periods of at least three consecutive days with daily mean air temperature above the 95th percentile of the all-season temperature distribution.

RESULTS: We will shown the results from first method in this abstract. The number of heat waves for thirty years in Novi Sad is illustrated in Figure 1. It can be seen that the highest numbers of tropical days ($\geq T_{max} 30.0^{\circ}C$) was observed in 1985 (4 days), 1990 (5,5 days) and 2000(6 days). As we see from 1984. year heat waves are frequent and intense. More interesting is heat waves per seasons, which is shown in Figure 2. As we can see heat waves are more common during the winter season.

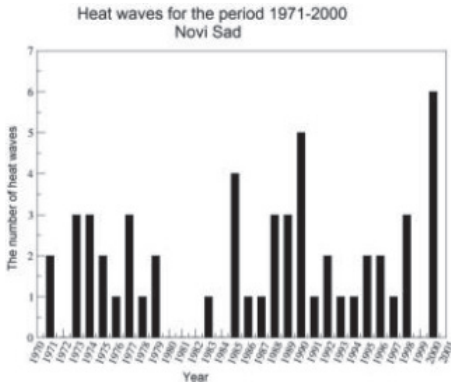


Figure 1: Heat waves for the period 1971-2000 for Novi Sad

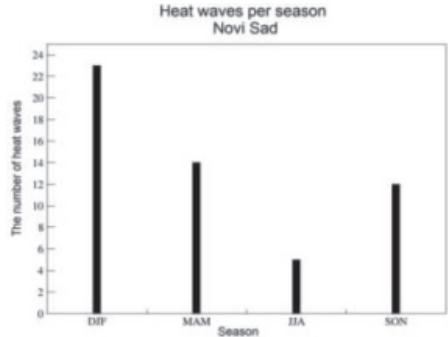


Figure 2: Heat waves per season for Novi Sad

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CONCLUSIONS:

In this paper, frequency and duration of heat waves and are presented for the period 1961-2000. The following points can be made:

- A very low occurrence of heat waves was typical in the 1980s and early 1990s.
- The highest frequency of the shortest and the weakest heat waves, while the longer and stronger waves occurred fewer times.
- The longest heat waves in Novi Sad were recorded in 1994 (*HWDI* =6 days) and in 2000 (*HWDI* =7 days).





T2

ABSTRACTS

Track 2: Physiology of living organisms





Nebojša Andrić

ENDOCRINE DISRUPTORS AND REPRODUCTION: MECHANISMS OF ACTION IN STEROID HORMONE-PRODUCING CELLS

Nebojša Andrić¹

¹University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia

*Corresponding author: nebojsa.andric@dbe.uns.ac.rs

KEYWORDS: Endocrine disruptors (EDs); fertility; steroidogenesis; nuclear receptors; signaling.

INTRODUCTION: There is growing interest in the possible health threat posed by endocrine disruptors (EDs), which are substances in our environment, food and consumer products that interfere with hormone biosynthesis, metabolism or action, resulting in a deviation from a normal homeostatic control or reproduction. Studies report rise in reproductive diseases and decline in reproductive function since the mid-20th century among certain locations and populations (primarily in the developed world). It has been estimated that ~15% of the population in industrially developed countries is affected by infertility. The number of risk factors that affect reproductive potency has grown in the last several decades. EDs have been identified as one of the potential risk factors that may be contributing to the observed changes in reproductive health. Increasing number of EDs is found in human amniotic fluid including pesticides, plasticizers, flame retardants, etc. Accumulating evidence indicate that EDs can interfere with steroidogenic process in the gonads, thus exerting the potential to interfere with fertility. Recent studies provide the mechanisms of action of EDs on steroidogenesis. These involve actions through the nuclear receptors, such as estrogenic, androgenic, thyroid, peroxisome proliferator-activated receptor γ , retinoid receptors, as well as through different signaling pathways, steroidogenic enzymes, neurotransmitter receptors and systems, and many other pathways that are highly conserved in wildlife and humans. It would be presented and discussed the data showing that some EDs including pesticides (atrazine), plasticizers (bisphenol A) can interfere with signaling steroid hormone-producing cells, thereby affecting steroidogenesis and fertility.

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ESTABLISHMENT OF CELL-BASED ELISA FOR QUANTITATIVE MEASUREMENT OF ENVIRONMENTAL CHEMICAL-INDUCED ERK1/2 ACTIVATION IN HEPG2 CELLS

Bojana Stanic¹, Jelena Hrubik², Vivian Lu Tan³, Kristin Schirmer³, Nebojsa Andric²

¹ UNSFTN, Department of Environmental Engineering and Occupational Safety and Health, Novi Sad, Serbia,

² UNSPMF, Department of Biology and Ecology, Novi Sad, Serbia,

³ Eawag, Department of Environmental Toxicology, Dübendorf, Switzerland

*Corresponding author: nebojsa.andric@dbe.uns.ac.rs

KEYWORDS: ELISA; ERK1/2; HepG2; environmental chemicals

INTRODUCTION: Traditional chemical risk assessment studies rely primarily on a complex set of whole-animal-based toxicity testing strategies for hazard identification. These studies exert difficulties in addressing a wide variety of challenges that toxicology meets today, including ethical concerns due to a large number of animals used for these studies and inability to define the molecular mechanisms by which chemicals exert their toxic action. Therefore, development of new toxicological tests that will allow for more efficient characterization of chemical-organism interactions is warranted. Exploring perturbations in cellular signaling pathways in response to chemical stress using cultured cells is an emerging area of toxicological research. Environmental chemicals can interact with these pathways, thus altering cellular homeostasis, leading to dysfunction. This feature makes signaling pathways excellent indicators of exposure identification and hazard prediction. Extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway has recently been recognized as an important component of cellular response to a range of non-physiological stimuli, including various environmental chemicals.

OBJECTIVES: Establish and optimize the phosphospecific ERK1/2 enzyme-linked immunosorbent assay (ELISA) on human hepatocellular carcinoma cell line HepG2 in 96-well plates and validate the assay's capacity to detect ERK1/2 signal originating from structurally diverse compounds with different chemical properties and modes of action.

METHOD / DESIGN: HepG2 cells were seeded into 96-well tissue culture plates and serum-deprived for 24 h prior to stimulation with either the classical ERK1/2 activator epidermal growth factor (EGF) in a range of concentrations (from 0.1 to 1000 ng/mL) or the test compounds (tributyltin, TBT; bisphenol A, BPA; benzo-a-pyrene, BaP; atrazine, ATR; ibuprofen, IBU) in a range of concentrations (from 10⁻⁵ to 10⁻¹² M) during different time points. The cells were fixed, permeabilized and the endogenous peroxidase was quenched, followed by blocking and incubation with p-ERK1/2 primary antibody overnight at 4°C. Next day, the cells were incu-

bated with the peroxidase-conjugated secondary antibody, followed by addition of o-phenylenediamine substrate solution. The cells were subsequently stained with crystal violet for cell number estimation.

RESULTS: Assay optimization was performed using EGF and implied establishing the optimal seeding density (2×10^4 cells/well), selecting the blocking solution (5% bovine serum albumin) and the optimal concentration of the primary and the secondary antibody (1:1000 dilution for both). Next, we analyzed the ERK1/2 response after exposure to selected environmental chemicals. The results showed that the highest doses of TBT (10^{-5} and 10^{-6} M) activated ERK1/2 in early time points (15 and 30 min); however, p-ERK1/2 signal decreased sharply below the control value after prolonged incubation (60, 120 and 360 min) due to the cytotoxic effect of this compound. TBT at the doses of 10^{-10} , 10^{-9} and 10^{-7} M caused a significant rise in ERK1/2 activity after 120 min exposure. BPA caused statistically significant ERK1/2 activation only at the 10^{-9} M dose during 15 min exposure. Fluctuations in p-ERK1/2 signal after ATR exposure never reached statistical significance. BaP and IBU caused a significant ERK1/2 activation only at the lower doses (10^{-11} to 10^{-7} M) during short exposure periods (15 and 30 min), whereas the higher doses were without effect. The assay revealed that all investigated chemicals, except ATR, perturbed ERK1/2 signaling pathway, suggesting that these chemicals can potentially disrupt various ERK1/2-regulated physiological processes in the cells.

CONCLUSIONS: In this study, we established an effective cell-based assay on HepG2 for measuring ERK1/2-disrupting effect of environmental chemicals. Obtained results indicate that ELISA on fixed cells has the potential to become a signaling-based method of choice for fast processing of a large number of samples in order to assess the toxic effect of environmental chemicals. Additional research, including mathematical modeling and testing of a larger number of environmental chemicals, is necessary before cell-based ELISA could be routinely used in toxicological research.

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TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF *ARION VULGARIS* – PROTEINS FOR PROBABLY SUCCESSFUL SURVIVAL STRATEGIES?

Tanja Bulat¹, Roman Smidak¹, Fernando J. Sialana¹, Gangsoo Jung¹,
Thomas Rattei², Martin Bilban³, Helmut Sattmann⁴, Gert Lubec⁵, Jana Aradska¹

¹ Department of Pediatrics, Medical University of Vienna, Vienna, Austria,

² Division of Computational System Biology, Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria,

³ Department of Laboratory Medicine and Core Facility Genomics, Medical University of Vienna, Vienna, Austria,

⁴ Third Zoological Department, Museum of Natural History Vienna, Vienna, Austria,

⁵ Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria

*Corresponding author: tanja.bulat@gmail.com

KEYWORDS: *Arion vulgaris*; transcriptome; proteome; nano-LC-ESI-MS/MS analysis; Lectin-like proteins

INTRODUCTION: The Spanish slug, *Arion vulgaris*, is considered one of the hundred most invasive species in Central Europe. The immense and very successful adaptation and spreading of *A. vulgaris* suggest that it developed highly effective mechanisms to deal with infections and natural predators.

OBJECTIVES: Current transcriptomic and proteomic studies on gastropods have been restricted mainly to marine and fresh water gastropods. No transcriptomic or proteomic study on *A. vulgaris* has been carried out so far, and in the current study, the first transcriptomic data base from adult specimen of *A. vulgaris* is reported.

METHOD / DESIGN: The first mRNA-derived protein data base was constructed for protein identification. A gel-based proteomic approach was used to obtain the first generation of a comprehensive slug mantle proteome.

RESULTS: Total of 2128 proteins were unambiguously identified; 48 proteins represent novel proteins with no significant homology in NCBI non-redundant data base. Combined transcriptomic and proteomic analysis revealed an extensive repertoire of novel proteins with a role in innate immunity including many associated pattern recognition, effector proteins and cytokine-like proteins. The number and diversity in gene families encoding lectins point to a complex defense system, probably as a result of adaptation to a pathogen-rich environment.

CONCLUSIONS: The results are providing a fundamental and important resource for subsequent studies on molluscs as well as for putative antimicrobial compounds for drug discovery and biomedical applications.

DAILY DYNAMICS OF BEECH PHOTOSYNTHETIC PARAMETERS IS SIGNIFICANTLY DISTURBED BY PERIODICAL DROUGHT AND SPECIFIC MICROHABITAT CONDITIONS

Borišev Milan¹, Župunski Milan¹, Pajević Slobodanka¹, Rita Horak,³
Pilipović Andrej², Nikolić Nataša¹, Arsenov Danijela¹, Orlovic Saša²

¹ University of Novi Sad, Department of biology and ecology, Faculty of Sciences, Serbia

² University of Novi Sad, Institute of Lowland Forestry and Environment, Serbia

³ University of Novi Sad, Faculty of Education on Hungarian language, Subotica, Serbia

*Corresponding author: milan.borisev@dbe.uns.ac.rs

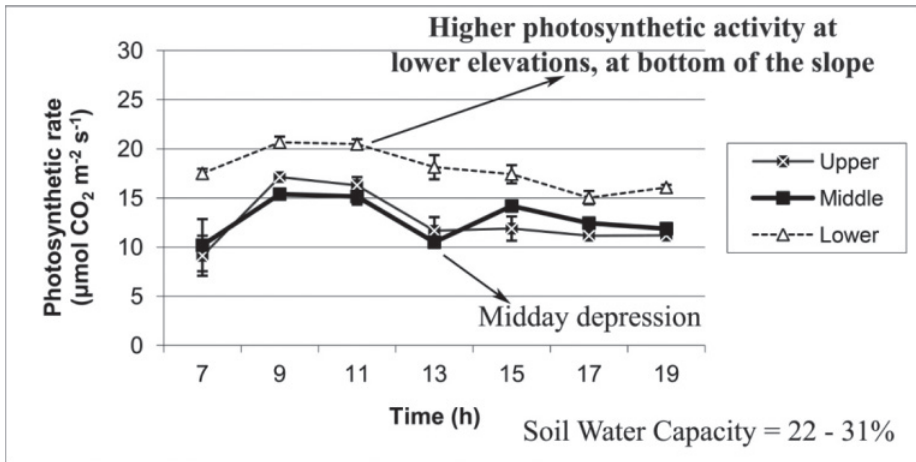
KEYWORDS: beech forest; drought impact; climate change

INTRODUCTION: In most parts of Europe climate change will be one of the main driving forces determining forest and agricultural plant yields, performance and stability. Low water availability is one of the main environmental factors influencing plant growth and yield in many regions of the world. Impact of periodic drought in beech populations of Fruška gora National Park can significantly disturb stable forest bioproductivity.

OBJECTIVES: Aim of this study was to analyze daily photosynthetic activity of beech populations in relation to drought occurrence and altitude of specific microhabitats.

METHOD / DESIGN: Three investigated beech populations were located on the same slope of the mountain, at the top of the hill ridge, middle of the slope and in the bottom next to a small mountain creek. Measurements of photosynthetic activity and parameters of plant and soil water regime were performed by portable photosynthesis system LCpro+ ADC BioScientific, every month during the vegetation season, every two hours from 7 to 19h during the day.

RESULTS: Our analyses of photosynthetic activity indicate that lack of precipitation can seriously reduce beech trees photosynthetic activity, mostly on upper ridge and peak localities with quick water runoff. Consequently lack of soil humidity develops much faster, than in the lower parts of the slope. At first, initial influence of water deficit induced a significant decrease in the afternoon photosynthetic activity, with increase of water use efficiency. At the same time, CO₂ assimilation shows strong midday depression. Continuous dry conditions, lasting up to several weeks, cause complete reduction of the afternoon activity. Thus, stomata are active only during the first part of the morning, with sufficient CO₂ assimilation until 10-11 a.m., reducing light use efficiency only to 3-4 hours during the day.



Average photosynthetic activity of beech on upper, middle and at lower part parts of the mountain slope

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CONCLUSIONS:

Results of this research emphasize the importance of microhabitat parameters in beech adaptations to drought induced by unstable climate, especially considering that water limitations are an increasingly important factor in future plant bioproductivity.



VEGETABLES QUALITY ASSESSMENT ON THE BASIS OF NITRATE, NITRITE AND HEAVY METAL CONCENTRATIONS

Slobodanka Pajević¹, Nataša Nikolić¹, Milan Borišev¹, Dejan Orčić¹, Neda Mimica-Dukić¹, Danijela Arsenov¹ and Milan Župunski¹

¹University of Novi Sad Faculty of Sciences, Trg Dositeja Obradovića 3. Novi Sad, Srbija

*Corresponding author: slobodanka.pajevic@dbe.uns.ac.rs

KEYWORDS: vegetables; contamination; lead; cadmium; chromium; nickel; nitrates; nitrites.

INTRODUCTION: Introduction: Heavy metals (HM) and other harmful substances mostly enter into the human body through a chemically contaminated food and water. HM, as well as nitrites and nitrates which are the most common inorganic nitrogen constituents of plant tissues, not only reduce the quality of food, but they are extremely harmful when introduced into the human body. Therefore, continuous monitoring of the chemical composition of plants important in human nutrition and the quality of the soils on which they are grown is of high priority.

Plants have the capacity to absorb and accumulate heavy metals and pollutants which indicated their characteristic as bio-indicators. Therefore, according to the chemical composition of the plant tissue, it could be very reliably evaluated the quality of growing practice and applied agricultural technology. Based on these results, it could be suggested to the vegetable producers how to implement (and reduce) fertilization, application of pesticides and herbicides, move the agricultural plots away from roads and polluted environment etc.

OBJECTIVES: Although it would be desirable, it is not always possible to accomplish quality control of vegetables ranging from farm to final product, because of the large number of individual manufacturers. Therefore, the basic idea and goal of this study was to examine the quality of the vegetable foods that reach consumers depending on locality of growing.

METHOD / DESIGN: Samples of the most abundant vegetable species in human nutrition were collected from the Novi Sad green markets, in 2009, 2010, 2011, 2012. Vegetable originated from producers who cultivated vegetables in different locations in Vojvodina and Novi Sad area.

The edible plant parts are prepared for chemical analysis by standard procedures. Heavy metals determination was done by atomic absorption spectrophotometry and their concentrations in plant tissue were expressed in µg/g dry mass. The results were processed statistically by method of analysis of variance (ANOVA). The level of vegetable pollution has been determined according to the "Regulations for pesticides, metals and metalloids and other toxic substances, hemotherapeutics, anabolic steroids and other substances which can be found in food (Sl. list SRJ", br. 5/92, 11/92 – ispr.

i 32/2002 i Sl. glasnik RS”, br. 25/2010 – dr. pravilnik i 28/2011 – dr. pravilnik) where the maximum permissible levels (MPC) of Cd and Pb in dry vegetables has been set to 0.3 and 3 mg/kg, respectively, whereas for chromium limits are not defined. At the same time, MPC values recommended by FAO/WHO organization (Food additives and contaminants. Joint Codex Alimentarius Commission, FAO/WHO Food Standards Programme, ALINORM 01 12A. 2001), are 0.2 mg/kg for Cd, 2.3 mg/kg for Cr, 0.3 mg/kg for Pb.

RESULTS: The lowest average value of the concentration of cadmium was detected in the inflorescence of cauliflower (0.12 µg/g), and highest in spinach leaves (0.48 µg/g) which is above the MPC. High average values of Cd were detected in carrot roots (0.29 µg/g) and tomato fruits (0.27 µg/g). The lowest average concentration of nickel was detected in the red beet (0.75 µg/g), and highest in spinach leaves (2.19 µg/g). The lowest average concentration of lead has been found in the root of parsnip (1.11 µg/g), followed by potatoes (1.13 µg/g), and highest in spinach leaves (3.56 µg/g) which was above the MPC. We detected the highest concentration of nitrate in red beet (average 16.7 mg/ g), and the highest nitrite content in spinach leaves (1030.0 µg/g).

CONCLUSIONS: The content of the tested contaminants in vegetables varied considerably depending on plant species and growing location. Many vegetable samples contained disturbingly high levels of investigated metals. The level of cadmium exceeded the MPC values in 16.8% of the total number of samples, while the lead content exceeded the MPC values in 16.1% of the investigated vegetable species.

THE FUNGICIDE PYRIMETHANIL CAUSES TESTICULAR ALTERATIONS AND SPERMATOTOXICITY IN ADULT MALE SPRAGUE-DAWLEY RATS

Margareta Nikolić¹, Milena Aleksić¹, Andrea Žabar Popović¹,
Jelena Conić¹, Perica Vasiljević¹

¹ Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš,
Višegradska 33, Niš, Serbia

*Corresponding author: nmara27@gmail.com

KEYWORDS: Fungicide; pyrimethanil; reproductive; toxicology; spermatogenesis.

INTRODUCTION: It is known that fungicides show large variations in their potential to cause toxic effects in animals, arising from the use of fungicides in agriculture and fruit production. Pyrimethanil (4, 6-Dimethyl-N-phenyl-2-pyridinamine) is an anilinopyrimidine fungicide. It belongs to organic group of systemic fungicides, which act as a contact, surface fungicide or it is moving translaminar through the plant. Pyrimethanil is preventing plant diseases caused by a broad spectrum of fungi.

Concentrated suspension of pyrimethanil (400 g/l) Pyrus 400 SC, used in this study, is commercially used for suppression of gray mold of grapevine (*Botrytis cinerea*), sooty leaf spot in vines and raspberry (*Rubus sp.*) and apple scab (*Venturia inaequalis*).

OBJECTIVES: Based on previous findings that fungicides have toxic effects on the male reproductive system, and the fact that pyrimethanil has great commercial use in fruit production and vineyards, it is important to examine what are the consequences of use of this fungicide on the reproductive system in mammals, which are still unknown. Therefore this study was designed to investigate spermatotoxic effects and the ability of this fungicide to induce morphological changes in seminiferous tubules of testes in Sprague-Dawley rats treated orally with different doses of pyrimethanil.

METHOD / DESIGN: This study was designed to analyze and define the effects of 5-day oral administration of six different concentration of concentrated pyrimethanil suspension (Pyrus 400 g/l) on adult, 12 weeks old, male Sprague-Dawley rats, randomly divided into seven groups (3 per cage), maintained at 22±2°C, at 12/12h light and dark cycle. Food and water were provided *ad libitum*. In six experimental groups rats were orally treated with Pyrus dissolved in PBS at dosages of 500mg/kg, 600mg/kg, 700mg/kg, 800mg/kg, 900mg/kg and 1g/kg, while control group was treated only with PBS. After 5- day treatment, rats were anesthetized with 0, 3ml 10% ketamidol. Immediately after sacrifice, epididymis of each animal was used for determination of motility of spermatozoa. After excision, testes were fixed in 4% paraformaldehyde and sectioned at 2µm after paraffin embedding. Sections were stained with hematoxylin and eosin and then examined on lightmicroscope (Leica DM2500) for analyses of different morphometric characteristics: number of spermatogenic and Sertoli cells/in the field of view, cell area and nucleus area, luminal area of seminiferous tubules and

also area of intercellular space between spermatogenic cells. Statistical analysis was performed using the statistical software package “SYSTAT13”.

RESULTS: After treatment, results showed that sperm motility was absent in 700mg/kg, 800mg/kg, 900mg/kg and 1g/kg groups, while it was present in control group and groups with lower concentrations of pyrimethanil applied. Statistical analysis of different morphometric characteristic showed that *in vivo* treatment with pyrimethanil reduce level of spermatogenic cells in the seminiferous tubules in dose-dependent manner. There was a significant reduction in the number of secondary spermatocytes after treatment with the highest concentration of pyrimethanil (1g/kg), from 100.1 ± 18.2 cells/field of view (control) to 13.3 ± 11.4 cells/field of view. Also pyrimethanil caused a significant decrease in the area of spermatogenic and Sertoli cells and decrease in their nucleus area, gradually from control group to 1g/kg group. It was obtained significant decrease in area of Sertoli cells from $132.7 \pm 30.02 \mu\text{m}^2$ in control group to $63.6 \pm 8.6 \mu\text{m}^2$ in 1g/kg group. There were morphological changes of seminiferous tubules. The results showed a reduction in the luminal area, where effect of the highest concentration (1g/kg) was drastic reduction of $8,831.718 \pm 2,385.364 \mu\text{m}^2$ compared to control group with luminal area $17,356.616 \pm 5,972.545 \mu\text{m}^2$. The opposite effect was significant increase of intercellular space between spermatogenic cells in dose-dependent manner, from the 500mg/kg group to the group treated with highest concentration of 1g/kg.

CONCLUSIONS: In this experiment, results showed that pyrimethanil caused large number of pathological changes in the testes. First of all, we conclude that pyrimethanil leads to a reduction of reproductive (spermatogenic) capacity in males. The presence of necrotic cells and a significant reduction in the number of the cells indicate a proapoptotic effect of this fungicide. There was atrophy of seminiferous tubules, and the possible penetration and accumulation of fungicide in the interior of the tubules. In addition, there were observed cases of germ cells dissociation from the base of seminiferous tubules and their accumulation within the lumen. Based on previous research, conclude is that there may be ability of pyrimethanil to affect the function of Sertoli cells cytoskeleton, which impairs the adhesion of immature germ cells and Sertoli cells, which led to premature separa

EFFECT OF CADMIUM ON PHYSIOLOGICAL CHARACTERISTICS AND TOLERANCE OF *POPULUS DELTOIDES* L. AND *POPULUS X EURAMERICANA* CLONES

Nataša Nikolić¹, Slobodanka Pajević¹, Milan Borišev¹, Danijela Arsenov¹, Milan Župunski¹, Saša Orlović², Andrej Pilipović²

¹ Department of Biology and Ecology, Faculty of Sciences, Trg D. Obradovića 2, Novi Sad, Republic of Serbia

² Institute of Lowland Forestry and Environment, Antona Čehova 13, 21000 Novi Sad, Republic of Serbia

*Corresponding author: natasa.nikolic@dbe.uns.ac.rs

KEYWORDS: cadmium; phytoextraction; poplars; tolerance; toxicity

INTRODUCTION: Contamination of the environment with heavy metals is a world-wide problem. Cadmium (Cd) has attracted considerable scientific attention due to its toxicity to most living organisms, and its relative mobility in the soil–plant system. The use of fast growing woody plants for extraction of cadmium from contaminated soils appears a promising method for remediation of low to medium polluted soil. Poplars are good candidate species because of their rapid growth rate, high biomass yield, adaptability and genetic variability.

OBJECTIVES: This study was aimed at evaluating phytoremediation potential of *Populus x euramericana* and *Populus deltoides* clones through analysis of plant growth responses and tolerance, Cd accumulation, translocation, and partitioning between plant organs, and responses of morphological and physiological characteristics, and their relation with biomass production.

METHOD / DESIGN: Plants were cultivated in soil moderately contaminated with Cd, under semi-controlled conditions, for six weeks. For both clones, two treatments (control – 0 mg kg⁻¹ of Cd, and Cd treatment – 8.14 mg kg⁻¹ of Cd) were set up with four replicates. After 6 weeks of cultivation, morphological mass and physiological characteristics were measured, prior to harvesting of plants for chemical analysis. Concentration of Cd was measured in leaves, stems and roots.

RESULTS: The obtained results suggest that *Populus x euramericana* and *P. deltoides* clones differentially responded to Cd treatment. The treatment more negatively affected biomass production and morphological characteristics in *Populus x euramericana* than in *P. deltoides* plants. Photosynthetic characteristics and gas exchange parameters were affected by the treatment in most cases, but the level and pattern of changes depended on the clone. High tolerance to the applied Cd levels was observed in both clones, but it was higher in *P. deltoides* than in *Populus x euramericana*. We suppose that higher tolerance to Cd toxicity observed in *P. deltoides* could be related to unchanged proline content and undisturbed nitrogen metabolism.

CONCLUSIONS: *P. deltoides* showed better phytoextraction potential and performance under Cd exposure than *Populus x euramericana* clone, suggesting its potential not only for phytostabilization, but also for phytoextraction projects. The screening based on physiological and morphological plant characteristics might be considered an efficient method for evaluating the crop performance and tolerance to heavy metal stress.



ABSTRACTS

Track 3: Biotechnology, bioengineering and biosensing

T3





Wilaiwan Chotigeat

EXTRA-FUNCTION OF RIBOSOMAL PROTEIN L10A

Wilaiwan Chotigeat^{1,2,*}, Monwadee Wonglapsuwan^{1,2},
Kunwadee Palasin^{1,2}

¹ Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand,

² Center for Genomics and Bioinformatics Research Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla

*Corresponding author: wilaiwan58@hotmail.com

KEYWORDS: Ribosomal protein; extra-function; RpL10A, oogenesis; spermatogenesis

INTRODUCTION: Ribosomal proteins are generally known as a component of ribosome whereby protein synthesis is occurred. Recently, several ribosomal proteins have been reported on other functions excluding from protein synthesis. Ribosomal protein L26 (RpL26) was reported as a phagocytosis activating protein in shrimp and fish. A ribosomal protein S2 (RPS2) mutant was shown a block in development of oogenesis in fruit flies. Ribosomal protein S29 (RPS 29) can induce apoptosis in lung cancer cells, whereas Ribosomal protein L39a (RPL39a) and Ribosomal protein S9 (RPS9) are protectors against cell death. Ribosomal protein L10A (RpL10A) was demonstrated higher expressed during vitellogenic stage of shrimp. Therefore it is curious to know whether RpL10A has extra-function on oogenesis in shrimp and *Drosophila melanogaster* while the function on spermatogenesis was investigated in shrimp and mouse.

OBJECTIVES: To study the effects of RpL10A on oogenesis and spermatogenesis

METHOD / DESIGN:

1. RpL10Ab⁻ follicle cell clones and germ line clones of *Drosophila* were generated by the FLP-FRT technique to investigated oogenesis in *Drosophila sp.*
2. Recombinant protein RpL10A was injected into shrimp to determine ovarian maturation in shrimp.
3. Recombinant protein RpL10A was incubated with the testis explants to stimulate spermatogenesis in shrimp and mice.

RESULTS: RpL10A was found to be essential for oogenesis in *Drosophila*. RpL10Ab homozygous mutants are lethal and germ line clone analysis showed premature death of the follicle cells, while the recombinant RpL10A (rRpL10A) promoted ovarian maturation in shrimp. In addition, the rRpL10A was examined as a stimulant for testicular development in shrimp. After rRpL10A protein incubation at 4 h, percentages of testicular stages were calculated. In this report, we classified spermatogenesis into 3 stages according to a diameter of cell under histological observation thereby stage I (spermatogonia cells, diameter = > 6.25 μm), stage II (spermatocyte I and II, diameter = 3.00-6.25 μm) and stage III (spermatid and spermatozoa, diameter = < 3.00 μm). The result showed that all groups of the rRpL10A (0.5, 1.0, 1.5 and 2.0 μM) stimulated spermatogenesis into stage III (mature stage). From the result, 1.5 μM of rRpL10A was the effective dose for testicular development in shrimp. In addition, stimulating of the rRpL10A on mouse testis was examined *in vitro*. The results showed the effect dose of RpL10A protein for mice was 1.0 μM . The results indicated that RpL10A protein induced development of spermatogonia into spermatid cells. Therefore, rRpL10A protein can promote spermatogenesis in both of shrimp and mouse.

CONCLUSIONS: In conclusion, the RpL10A is not only essential for protein synthesis in living organism but also have extra functions in specific tissues, for example oogenesis in ovary, spermatogenesis in testis. The knowledge gained from these works may apply to use for human better living in the future.



Vladimir Crnojević

**BIOSENSE INSTITUTE AND THE FUTURE
OF AGRICULTURE – DATA IN ACTION**

Vladimir Crnojević¹

¹ *BioSense Institute, University of Novi Sad*

**Corresponding author: crnojevic@biosense.rs*

KEYWORDS: Multidisciplinary research, micro and nanoelectronics, communications, signal processing

Research and Development Institute for Information Technologies in Bio- systems, also known as the BioSense Institute, has been founded to focus multidisciplinary, game-changing and needs-driven research and disseminate it to a global ecosystem of forward-looking stakeholders. Multidisciplinary research is performed in the fields of micro and nanoelectronics, communications, signal processing, remote sensing, big data, robotics and biosystems, with a common goal to cross-fertilize various bio-fields with ICT. BioSense promotes data science in various traditional bio-systems by solving different problems, difficult to comprehend with traditional knowledge – smart choice of seeds in agriculture, clustering of gene sequences, AIDS breakout modeling through mobile subscribers behavior etc. Putting the data in action to make the real benefit for biosystems - the real mission of BioSense Institute.



THE RNAi MACHINERY OF COLLEMBOLA

Phutita Wongwaiyut¹, Patamarerk Engsontia¹

¹ *Molecular Ecology and Evolution Research Unit, Department of Biology, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand*

*Corresponding author: patamarerk.e@psu.ac.th

KEYWORDS: RNAi; collembola; *Folsomia candida*; *Pogonognathellus longicornis*

INTRODUCTION: RNA interference (RNAi) is a conserved molecular process in eukaryotes which serves two important roles: gene regulation and defense against viruses and transposable elements. RNAi has been extensively studied due to its powerful applications in medical treatment and in functional genomic research (loss-of-function study/ gene silencing technique). In animal, three major RNAi pathways have been reported: siRNA, miRNA and piRNA pathways. The short interfering RNA (siRNA) pathway is the important mechanism to eliminate exogenous double-stranded RNA (dsRNA) (from viruses or experimentally introduced dsRNA). The microRNA (miRNA) pathway is vital for post-transcriptional control of gene expression. The piwi-interacting RNA (piRNA) pathway targets and eliminates the transposable elements. Major proteins involved in the RNAi pathways are Dicer proteins (Dcr-1, Dcr-2) that cut dsRNA into shorter pieces, Drosha protein for forming premature microRNA in the nucleus, Argonaute protein family (Ago-1, Ago-2, Ago-3, Aubergine, Piwi, Wago) and other proteins (Armitage, Spindle E, C3PO), which form the RNA-induced silencing complex (RISC) that bind to the mRNA and digest them, thus inhibiting translation. In addition, there are associated proteins for binding dsRNA (Loqs, R2D2, Pasha), protein for transporting dsRNA between cells (Sid-1), and RNA-dependent RNA polymerase (RdRP) for amplifying siRNA within cells. Understanding the variability of RNAi pathways is crucial for the development of therapeutic RNAi methods or function genomic tools. Collembola is the basal lineage of hexapod and key species for understanding the adaptation during the transition of aquatic/marine crustacean to a common ancestor of terrestrial hexapods. Investigating the RNAi machinery of collembola may provide new insight into the animal RNAi pathways.

OBJECTIVES: To identify genes involved in the RNAi pathways of collembola, *Folsomia candida* and *Pogonognathellus longicornis*, using transcriptome analysis.

METHOD / DESIGN: We constructed *F. candida* and *P. longicornis* transcriptomes from RNA extracted from the whole body (*F. candida*) or head (*P. longicornis*). The extracted RNA were converted to cDNA and sequenced using Illumina HiSeq 2000 platform. Paired-end reads (100 bp) were assembled into 138,315 and 442,820 contigs (*F. candida* and *P. longicornis*, respectively) using Trinity. The transcriptome databases were made on a PC using BLAST+ 2.2.31. We collected proteins involved in the insect RNAi pathways (mainly from fruit fly - *Drosophila melanogaster* and bed bug - *Rhodnius prolixus*) from the NCBI database and used them for the tBLASTn search (E

value = 10) against the collembolan transcriptome databases. Putative genes from collembola were BLAST checked against the NCBI database. Only sequences that show significant similarity to proteins involved in the RNAi pathways of other animals were kept in the final results.

RESULTS: Major proteins in RNAi pathways can be identified from both collembolan species but the numbers of gene vary. For *F. candida*, we identified Dcr-1 (2 genes), Dcr-2 (3 genes), Drosha (3 genes), Ago-1 (3 genes), Ago-2 (5 genes), Ago-3 (2 genes), Aubergine and Aubergine-like (Piwi and Wago) (11 genes), Armitage (1 gene), Spindle E (3 genes), C3PO (1 gene), Loqs (2 genes), R2D2 (1 gene), Pasha (1 gene) and Sid-1 (4 genes). For *P. longicornis*, we identified Dcr-1 (14 genes), Dcr-2 (6 genes), Drosha (6 genes), Ago-1 (3 genes), Ago-2 (6 genes), Ago-3 (4 genes), Aubergine and Aubergine-like (Piwi and Wago) (6 genes), Armitage (2 gene), Spindle E (5 genes), C3PO (3 gene), Loqs (3 genes), R2D2 (2 genes), Pasha (1 genes), Sid-1 (8 genes), and RdRP (3 genes). The presence of these proteins suggests that all three RNAi pathways (siRNA, miRNA, piRNA) are functional in collembola. The presence of Sid-1 proteins also suggests that systemic RNAi works in collembola. The most interesting feature of the collembola RNAi machinery is perhaps multiple gene duplications observed on many genes (e.g. 14 Dcr-1 and 8 Sid-1 in *P. longicornis*). The exposure to various viruses in the evolution of collembola might have shaped the evolution of their RNAi machinery. Roles of these duplicated proteins are unknown. They may function interchangeable leading to flexibility of RNAi pathways or, alternatively, they may interact to each other leading to more complexity of the pathways.

CONCLUSIONS: We have identified key RNAi-related proteins in collembola, *Folsomia candida* and *Pogonognathellus longicornis*. To our knowledge this is the first report of the entire RNAi machinery of this animal lineage. Our results suggest that all miRNA, siRNA and piRNA pathways function in collembola. Further investigations of these proteins are essential for the understanding of how duplicated proteins function in the RNAi pathways.

DETERMINATION OF SOME ELEMENTS FOR TECHNO-ECONOMIC ENZYMATIC HYDROLYSIS OF CELLULOSE IN HYDROTHERMALLY PRETREATED SUGAR BEET SHREDS

Darjana Ivetić¹, Tatjana Đorđević¹, Mirjana Antov¹

¹ Faculty of Technology, University of Novi Sad, Blvd. Cara Lazara 1, 21 000 Novi Sad, Serbia

*Corresponding author: mantov@uns.ac.rs (Mirjana Antov)

KEYWORDS: enzymatic hydrolysis; cellulose; sugar beet shreds; pretreatment; β -glucosidase; material balance.

INTRODUCTION: Lignocellulosic biomass is widely available, low cost and renewable source of cellulose which could be transformed into many valuable products after depolymerization.¹ Before depolymerization of cellulose lignocellulosic biomass must be subjected to the pretreatment. Pretreatment should induce physical and/or chemical changes in material that should be beneficial for forthcoming enzymatic hydrolysis of cellulose.¹ Because of mass loss it is important to determine material balance of pretreatment for techno-economic evaluation of the process. During enzymatic hydrolysis, degradation of cellulose polymer to glucose occurs through cooperative actions of complex of cellulolytic enzymes. Among them, β -glucosidase is key enzyme for efficient hydrolysis since it hydrolyzes cellobiose which often inhibits other enzymes - cellobiohydrolases and endoglucanases. However, β -glucosidase activity in the cellulases complex obtained from the main fungal producers is generally insufficient. Thus, in order to prevent limitation of the overall hydrolysis rate by accumulated cellobiose, β -glucosidase often must be supplemented to the cellulases' reaction mixture.² High cost of enzymes, generally, makes the supplementation of β -glucosidase another important element for the techno-economic evaluation of enzymatic hydrolysis of cellulose in pretreated lignocellulose material which needs to be optimized.

OBJECTIVES: The aim of this study was to define material balance of hydrothermal pretreatment of sugar beet shreds at different solid:liquid ratios and to optimize supplementation of β -glucosidase for the enzymatic hydrolysis of cellulose as the part of the determination of elements for techno-economic evaluation of the process.

METHOD / DESIGN: Sugar beet shreds were hydrothermally pretreated at three different values of solids to liquid ratio in pretreatment mixture (1:20, 1:30 and 1:40) providing three substrates for hydrolysis. Enzymatic hydrolysis of obtained substrates was conducted at fixed cellulases dosage (20 FPU/g) while its yield was optimized using 3_2 factorial plan. Solids load (4.5%, 5.5% and 6.5%) and dosage of β -glucosidase (0, 0.25 and 0.50 U/g) supplementation were independent variables whose influence on hydrolysis yield during enzymatic reaction was investigated.

RESULTS: Hydrothermal pretreatment caused degradation of sugar beet shreds and solubilization of its polymers, resulting in 25% to 42% mass loss upon pretreatment,

while cellulose was well preserved. The highest loss in material mass was noticed for substrate pretreated at 1:40 solids to liquid ratio. In addition, pretreatment increased water retention value of obtained substrates for approximately 3 times. For substrate obtained by pretreatment at 1:20 solid:liquid ratio high hydrolysis yields could be achieved at solids load 5.8-6.3 % without β -glucosidase supplementation, while the highest predicted yield could be achieved at 6.4% solids load and 0.1 U/g β -glucosidase dosage. β -Glucosidase supplementation 0.15 U/g to reaction with substrate pretreated at 1:30 was optimal at 6.5% solids load. High yield of cellulose hydrolysis of substrate obtained by pretreatment at 1:40 solid:liquid ratio at all investigated solids loads could be achieved by supplementing β -glucosidase at dosage 0.27 U/g. However, considering process economy and efficiency optimal conditions should be 0.25 U/g β -glucosidase and 6.5% solids load.

CONCLUSIONS: Results revealed that optimal supplementation of β -glucosidase to the enzymatic hydrolysis of cellulose depends both on procedure of hydrothermal pretreatment of sugar beet shreds and substrate solids load. These elements as well as those arising from material balance of the pretreatment have to be taken into consideration in order to ensure efficiency and cost effectiveness of overall process of enzymatic hydrolysis of cellulose from sugar beet shreds.

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XANTHAN BIOSYNTHESIS IN LABORATORY BIOREACTOR ON EFFLUENTS FROM WHITE WINE PRODUCTION

Zorana Rončević¹, **Ida Zahović¹**, Bojana Bajić¹, Jovana Grahovac¹, Jelena Dodić¹

¹ Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*Corresponding author: idaidaza@gmail.com

KEYWORDS: Xanthan; Biotechnological Production; Bioreactor; Wine Industry; Effluents.

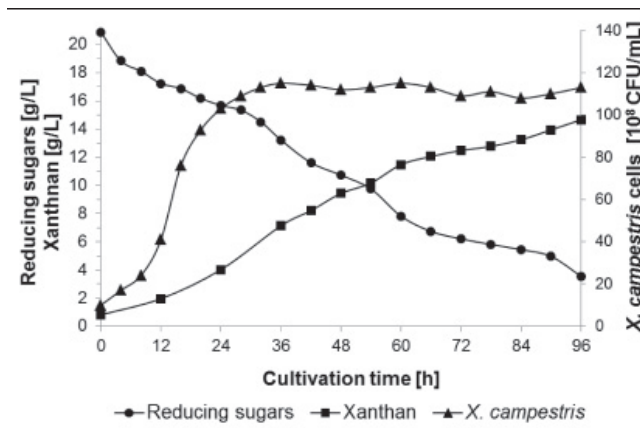
INTRODUCTION: Commercial wine production results in the generation of large amount of waste effluents. Wastewaters re major waste streams of wine industry which mainly originate from various washing operations that occur during the different stages of winemaking. These effluents typically contain large amounts of organic and inorganic contaminants as a result of product losses and applied cleaning practices. Also, winery wastewaters are characterized by large seasonal fluctuations in volume and composition, and are often discarded into environment with little or no treatment. The growing number of wineries and the demand for wine around the world are contributing to the increase this ecological problem. Therefore, the development of appropriate treatment methods is of crucial importance. There are numerous options available for the management of winery wastewaters which vary with respect to efficacy, cost and reliability. Among them, the biotechnological production of valuable products is one of the most promising alternatives for reduction the negative environmental impact and recycling the effluents generated during the wine production.

OBJECTIVES: The aim of this study was to examine the xanthan biosynthesis in laboratory bioreactor, using strain *Xanthomonascampestris* ATCC 13951, on liquid effluents from different stages of white wine production.

METHOD / DESIGN: Waste effluents from white wine production collected during the washing of the crusher, press and tank after clarification of must by flotation, were filtered and then analyzed in terms of sugar content. Based on obtained results, these wastewaters were mixed and adjusted to sugars content of 20 g/L, which is followed by addition of CaCO₃ in concentration of 2 g/L and correction of pH value. The xanthan biosynthesis was carried out in the 7 L laboratory bioreactor (Chemap AG CH-8604, Volketswil, Switzerland) under optimal conditions using reference strain *Xanthomonascampestris* ATCC 13951. The biomass growth, consumption of the sugar, nitrogen and phosphorus, xanthan formation, as well as rheological behavior of the medium were examined during the 96 h of cultivation.

RESULTS: The course of xanthan biosynthesis was examined by analyzing the cultivation broth samples in terms of parameters that are regularly used in observing the biotechnological process. The most significant results are shown on Figure 1. During

the cultivation rheological behavior of the medium was significantly changed, from the Newtonian at the beginning to very pseudoplastic in 96 h which is confirmed by values of consistency factor (2.5962 Pa·sn) and flow behavior index (0.3192). Process efficacy was estimated based on the raw xanthan yield (14.66 g/L) and degree of sugar conversion into product (70.21%). These results and residual content of sugars (3.55 g/L), total nitrogen (133.00 mg/L), assimilable nitrogen (2.80 mg/L) and phosphorus (2.82 g/L), as well as values of their conversion (82.98%, 48.65%, 95.65% and 85.11%, respectively) obtained in this research indicate that the xanthan production was successfully carried out in applied experimental conditions. Figure 1. Biomass growth, sugar consumption and xanthan formation during cultivation



CONCLUSIONS: Winery wastewaters, after additional optimization of the bioprocess in terms of the medium composition and the cultivation conditions, may be suitable substrates for industrial xanthan production. The use of these raw materials for xanthan biosynthesis would reduce the production costs and environmental problems caused by disposal of winery wastewaters.

BIOMECHANICAL AND BIOMIMETICAL PARAMETRIZATION OF TRUNK-DOMINATED TREES

Ivana Kovacic¹, Dragi Radomirovic², Miodrag Zukovic¹, Pavel Benka²,
Milutin Nikolic¹,

¹ University of Novi Sad, Faculty of Technical Sciences

² University of Novi Sad, Faculty of Agriculture

*Corresponding author: ivanakov@uns.ac.rs

KEYWORDS: tree; oscillations; frequency; damping; principle stiffness axes

INTRODUCTION: The scientific community working on the effects of excitations on trees is quite diverse: from botany and forestry to meteorology and biomimetics. From a mechanical point of view, trees have been recognized as blurring the boundary between a structure, material and mechanism. As such, they represent inspiring concept generators for improved or innovative solutions in engineering: resistant slender structures, adaptive dynamical systems and multifunctional applications. The diversity of interests mentioned and potential biomimetic applications are the basic motivating reasons for this pilot study, which is concerned with oscillatory response and mechanical characteristics of trunk-dominated trees.

OBJECTIVES: The objectives are: i) to determine values of basic oscillatory characteristics of a trunk-dominated tree; ii) to demonstrate the importance of principle stiffness axes and two frequencies associated with these axes, which have not been considered in Experimental Biology so far; iii) to show how oscillatory characteristics change along the trunk when the tree is in-leaf and out-of-leaf.

METHOD / DESIGN: Experimental investigations of the dynamic behaviour of a potted tree were carried out indoor by using the Vicon 3D motion capture system, which is a leading state-of-the-art infrared marker-tracking system. The object considered was a young trunk-dominated tree (*Aesculus hippocastanum*). Reflective markers were arranged along its trunk when it was in-leaf as well as when the leaves were removed. In both cases, pull-and-release tests were carried out and the motion was recorded. The 3D spatial displacements were then imported into a symbolic software package and the corresponding time-history diagram and trajectories of markers plotted and further analysed. Numerical and semi-analytical methodologies were developed to determine basic oscillatory characteristics: principle stiffness axes, frequencies of vibrations, as well as viscous damping ratios, which stem from aerodynamical and internal (material) damping.

RESULTS: Experimental measurements showed that markers arranged along the trunk, i.e. dots on the trunk, perform in-plane vibrations that have a beating character. Two methodologies developed (numerical and semi-analytical) gave consistent results about the values of the basic oscillatory characteristics of interest.

CONCLUSIONS: This pilot study points out the importance of principle stiffness axes and two close-valued frequencies associated with the beating oscillatory response of a trunk-dominated tree when performing free vibrations. It is calculated how they vary along the trunk. In addition, it is shown that the position of principle axis slightly changes when the tree is without leaves, while the change of the frequencies is significant. The variation of the viscous damping ratios was found to be more apparent along one principle axis only.

PRODUCTION OF BIOACTIVE SUGARS WITH HIGH ANTIOXIDANT POTENTIAL FROM AGRICULTURAL WASTE

Tatjana Đorđević¹, **Darjana Ivetić**¹, Mirjana Antov¹

¹ Faculty of Technology, University of Novi Sad, Blvd. Cara Lazara 1, 21 000 Novi Sad, Serbia

*Corresponding author: mantov@uns.ac.rs

KEYWORDS: bioactive sugars; antioxidant; wheat chaff.

INTRODUCTION: In recent years, agricultural residues have attracted great interest as one of the most promising sources for producing biofuels and biochemicals. One of them, wheat chaff, is abundant, low cost lignocellulose material which because of high carbohydrate content can potentially be used as feedstock for the production of some bioactive sugars. Functional sugars, in particular sugar oligomers, exhibit a range of biological activities including anti-infection, antimicrobial, anti-inflammatory properties as well as antioxidant activity (Moure et al., 2006; Christakopoulos et al., 2003). As many reports have observed the imbalance between pro-oxidants and antioxidants level related to pathogenesis of several chronic diseases, the investigation of new antioxidants from natural source is always attractive.

OBJECTIVES: The aim of this study was to investigate chelating ability and hydroxyl radical scavenging activity of liquid fractions obtained by ultrasound, alkali or hydrothermal treatment of wheat chaff.

METHOD / DESIGN: In this study, wheat chaff was used as raw material. Ultrasound, alkali or hydrothermal treatment of wheat chaff was applied. In all experiments, concentration of suspension was 10% (w/w) based on dry weight. Concentration of reducing sugars in samples was determined by DNS method with xylose as standard. In additions, sugars in liquid fractions obtained by treatments were analyzed by HPLC system (Waters, US) with RI detector using Zorbax column in isocratic mode at flow rate of mobile phase 1 mL/min (water:acetonitrile = 60:40 (v/v)). Antioxidant potential of obtained liquid streams-hydroxyl radical scavenging activity and chelating ability of ferrous ions (Fe₂₊) were assayed.

RESULTS: Pretreatments applied in this study caused release of different amounts of reducing sugars into the liquid fractions which was the consequence of differences in phenomena occurring during treatments as well as their different influence on particular constituents of wheat chaff. Qualitative HPLC analysis of the carbohydrate profile of liquid fractions indicated that predominant sugars were glucose and xylooligomers (triose, tetraose, pentose). Results indicated that all liquid fractions showed considerable antioxidant activity while fraction obtained by alkali treatment expressed the highest antioxidant potential. The results were expressed as EC₅₀ values which represent the effective concentrations of sample that is required to show 50% antioxidant properties indicating that lower value corresponds to higher antioxidant activity. EC₅₀ values for

metal chelating activity and hydroxyl scavenging of liquid fraction obtained by alkali treatment were 0.06 mg/mL and 0.43 mg/mL, respectively, being superior and equal, respectively, to antioxidant activities of standard compounds used in assays.

CONCLUSIONS: Results showed potential use of liquid fractions generated by different treatments of wheat chaff as a source of valuable products with high antioxidant potential.

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DECELLULARIZED HUMAN ADIPOSE TISSUE AS IN VITRO BIOMIMETIC MICROENVIRONMENT FOR CELL BEHAVIOR STUDIES AND DRUG TESTING

Drenka Trivanović¹, Tamara Kukolj¹, Hristina Obradović¹, Ivana Okić Djordjević¹, Jelena Krstić¹, Ivana Drvenica², Slavko Mojsilović¹, Aleksandra Jauković¹, Juan Francisco Santibanez¹, Diana Bugarski¹

¹ *Laboratory for Experimental Hematology and Stem Cells, Institute for Medical Research, University of Belgrade, Serbia*

² *Innovation Center of Faculty of Technology and Metallurgy, University of Belgrade, Serbia*

*Corresponding author: drenka.trivanovic@imi.bg.ac.rs

KEYWORDS: decellularization; adipose tissue; cancer cells; mesenchymal stem cells, microenvironment

INTRODUCTION: Biological scaffolds derived from decellularized tissues and organs have received significant attention in regenerative medicine, while the use of human decellularized adipose tissue extracellular matrix (hAdECM) shows additional great potential for soft-tissue regeneration and reconstitutive, corrective and cosmetic procedures. However, recent data pointed that adipose tissue grafts can stimulate the growth of residual cancer cells, especially when utilized in breast reconstruction surgeries due to the similar extracellular matrix composition to the microenvironment of breast tissues.

OBJECTIVES: The goal of this study was to evaluate the potential of hAdECM as biomimetic microenvironment to modulate the growth of both primary human adipose tissue-derived mesenchymal stem/stromal cells (hASCs) and breast cancer cells (MCF-7 and MDA-MB-231 lines), as well as for screening of cancer therapeutics.

METHOD / DESIGN: Human adipose tissue, obtained from healthy donors, was decellularized, lyophilized, solubilized and thereafter used for pre-coating of standard cell culture dishes. Oil Red O staining, which allows for the identification of adipocytes and lipid content, was used as direct way to assess decellularization. hAdECM was characterized for its ability to affect cells growth in comparison to tissue culture polystyrene (TCP) surfaces, collagen- and fibronectin-coated surfaces. Cellular metabolic activity and proliferation were determined by MTT or CFSE labeling, respectively, while the epithelial to mesenchymal transition (EMT), as a hallmark of tumor progression, was determined by immunofluorescent determination of epithelial (E-cadherin) and mesenchymal (Vimentin) markers expression.

RESULTS: The results demonstrated that hASCs exhibited similar growth rate and metabolic activity when cultured on all tested surfaces, although in comparison to the control TCP and collagen-treated surfaces, these cells exhibited more pronounced spindle-shape morphology and expression of Vimentin when cultivated in presence of hAdECM. The growth patterns of both breast cancer cell lines cultured on hAdECM

did not differ from the growth of those cells cultured on the other tested surfaces. However, when doxorubicin treatment was applied, in the presence of hAdECM, both cell lines demonstrated higher metabolic activity in comparison to the other surfaces, indicating that the presence of hAdECM significantly affects the antiproliferative effect of doxorubicin. Additionally, the hAdECM microenvironment promoted the epithelial to mesenchymal transition of both cell lines, by decreasing E-cadherin and increasing Vimentin expression.

CONCLUSIONS: The results indicate that hAdECM may provide more rational model to approach 3D specific culture systems for basic research studies of cell behavior and more importantly to provide (breast) cancer cells with a biomimetic microenvironment in vitro that can afford more meaningful information for the characterization of cancer cells growth and screening of cancer therapeutics.

CHARACTERISATION OF SCREEN-PRINTED TiO₂ NANOPARTICLES ON FLEXIBLE SUBSTRATE FOR HUMIDITY SENSING

Georges Dubourg¹, Marko Radović¹, Slavica M. Savić¹, George Niarchos¹, Goran Kitić¹, Tamara B. Ivetić², Massere Doumbia¹, Nikolina Janković¹ and Vesna Crnojević-Bengin¹

¹ Biosense Institute, University of Novi Sad, Serbia

² Department of Physics, Faculty of Sciences, University of Novi Sad, Serbia

*Corresponding author: georgesdubourg@uns.ac.rs

KEYWORDS: Nanomaterials; flexible substrate; humidity sensor; screen-printing.

INTRODUCTION: Printed electronics on flexible substrates using electrically functional inks is an emerging technology nowadays. Fabrication of printed electronic sensors has many advantages such as low-cost, lightweight, and being bendable, rollable, portable and foldable. In parallel, the increasing concern about environmental protection and food quality control has resulted in a continuous expansion in chemical sensor development and an important interest is now given to the production of cost effective, flexible, disposable and highly sensitive gas sensors. Although numerous types of printed chemical sensors have been established in agriculture, their introduction in the market has not yet been accomplished, and they are still remaining in the laboratories. A key parameter is the deposition of sensing materials that can be adapted to the printed electronics technological processes, such as printing methods since the active layer is usually deposited by spin-coating or micropipette drop casting, both of them methods which are not yet quite adapted for large-scale fabrication.

OBJECTIVES: The objectives of this work are the fabrication and characterization of chemical sensors using printed metal oxide nanoparticles as sensitive layers. Here, we present the production of humidity sensors using a screen-printing method for the deposition of TiO₂ nanoparticles.

METHOD / DESIGN: The humidity sensor consists of TiO₂ nanoparticles screen-printed on top of interdigitated electrodes (IDEs) patterned on flexible substrate. The design of the sensors is simple and does not require the high-cost semiconductor manufacturing equipment and high temperature steps normally used for silicon micro-fabrication. Initially, a gold layer was deposited by electron-beam evaporation on commercial PET substrate. Afterwards, the resulting layer was directly patterned by laser ablation using a short pulse laser (Nd:YAG-1064 nm, Rofin), in order to create micro-scale interdigitated electrodes. TiO₂ anatase nanoparticles (Sigma-Aldrich, particle size 21nm) were used as the sensitive layer. A paste was prepared by homogenizing TiO₂ nanopowder and ethanol in a Retsch PM100 planetary ball mill. The homogenization was performed in a continual regime using YSZ (yttrium stabilized zirconia) balls (2 mm in diameter) with the constant disc rotation speed of 300 rpm for 3h.

RESULTS: The gold electrodes of the resulting sensors are 50 μm in width and are separated by a gap of 50 μm . The sensitive layer area is characterized by a width of 1.5 mm and a length of 2mm. Preliminary resistive measurements of the humidity sensors were conducted in room temperature to test their responses on 100% relative humidity levels while measuring the changes in the resistance between the device's contact pads. A resistance change of two-orders of magnitude was observed for a relative humidity variation from 30 to 100%. Electrical characterization of devices with no active layer confirms that the obtained signal is mainly due to the presence of the TiO_2 nanoparticles. Response and recovery times of the sensor are relatively fast considering that no refreshment method is performed.

CONCLUSIONS: These results suggest that screen-printed TiO_2 nanoparticles are sensitive to humidity changes. Additionally, the low-temperature process proposed in this work can pave the way to large-scale fabrication of printed sensors on flexible substrate. Systematic characterization and sensor optimization results will be presented.

**MIXED MICELLES OF BINARY SURFACTANT MIXTURES:
TWEEN 40 – NA-3,12-DIOXO-5B-CHOLANATE;
TWEEN 80 – NA-3,12-DIOXO-5B-CHOLANATE AND THEIR
THERMODYNAMIC DESCRIPTION-CHARACTERISATION**

Mihalj Poša¹, **Stoja Obradović¹**, Ana Pilipović¹

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

*Corresponding author: stojaobradovic@gmail.com

KEYWORDS: Regular solution theory; Excess Gibbs energy; Bile salts; Tween 40; Tween 80

INTRODUCTION: The understanding of the nature and the characteristics of binary mixed micelles is vital in order to estimate the possibilities of their pharmaceutical and industrial application.

OBJECTIVES: Regular solution theory (RST) was compared with model independent thermodynamical methods (MIM) to analyse binary mixed micelles. The algorithm used to analyse the binary surfactant mixtures, i.e., the method for determination whether the binary mixture of the surfactants is symmetrical and obeys RST, was developed.

In order to investigate their stability, binary mixtures of Tween 40 - Na-3,12-dioxo-5 β -cholanate (T40-3,12-DOC) and Tween 80 - Na-3,12-dioxo-5 β -cholanate (T80-3,12-DOC) were analysed.

METHOD / DESIGN: Experimental values of critical micelle concentrations of Tween 40, Tween 80, - Na-3,12-dioxo-5 β -cholanate and their binary mixtures have been obtained by spectrofluorimetry using pyrene as a probe molecule.

RESULTS AND CONCLUSIONS: It was found that if the molar fractions of surfactants obtained using RST and MIM method do not differ, binary mixed micelles are symmetrical, according to the excess Gibbs energy.

Mixed micelles of T40-3,12-DOC were found to be more stable compared with the hypothetical ideal mixed micelles, i.e., they are primarily symmetrical, based on excess Gibbs energy, but they don't obey RST. Mixed micelles of T80-3,12-DOC were non-symmetrical according to excess free energy and less stable than the ideal mixed micelles. With the increase in the molar fraction of T80 in the mixed micelle, the micelle became less stable and decomposed into micelles of the building units (**Fig 1**).

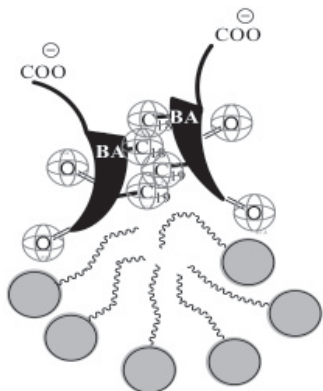


Fig 1. Mixed micelle decomposes to the building units

BINARY MIXED MICELLES BRIJ S10 - TWEEN 20 AND BRIJ S10 - TWEEN 60: THERMODYNAMIC DESCRIPTION

Stoja Obradović¹, Mihalj Poša¹

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

*Corresponding author: stojaobradovic@gmail.com

KEYWORDS: Regular solution theory; Excess Gibbs energy; Brij S10; Tween 20; Tween 60

INTRODUCTION: Nonionic surfactants Brij S10, Tween 20 and Tween 60 were studied in this paper. By calculating thermodynamic parameters (entropy, Excess Gibbs energy) we were able to assess the applicability of Regular solution theory on their mixed micelles, and therefore comprehend the nature of the micellization process and the deviations of real binary systems compared to the ideal ones.

OBJECTIVES: The influence of temperature and the length of the hydrophobic chain of Tween 20 and Tween 60 on the micellization process with Brij S10 was studied. By calculating additional Gibbs energy and analyzing the dependence $\beta=f(T)$, we investigated if entropy also contributes the Gibbs energy of micellization process, or if it is solely of enthalpic nature.

METHOD / DESIGN: Experimental values of critical micelle concentrations of individual surfactants and of their binary mixtures have been acquired by spectrofluorimetry using pyrene as a probe molecule.

RESULTS: For every analyzed system, with the rise in temperature, β parameter decreases. Obtained values of β and the values of molar fractions of individual surfactants in mixed micelles were used to calculate the excess entropy. Brij S10 – Tween 20 systems show higher values of excess entropy than Brij S10 – Tween 60 systems do (Table 1 and Table 2).

S^E/R				
T (K)\(\alpha	0.2	0.4	0.6	0.8
273.15	-0.022	-0.078	-0.138	-0.885
283.15	0.461	0.313	0.087	-1.024
293.15	0.572	0.296	0.178	-0.855
303.15	0.503	0.382	0.328	-1.14
313.15	0.866	0.625	0.795	7.45

Table 1. Entropy values for Brij S10/Tween 20 system.

S^E/R				
T (K)\(\alpha	0.2	0.4	0.6	0.8
273.15	-0.104	-0.189	-0.145	-3.04
283.15	-0.091	-0.354	0.034	-1.795
293.15	-0.071	-0.231	-0.015	-2.121
303.15	-0.06	-0.189	-0.083	-0.138
313.15	-0.026	-0.102	0.116	2.38

Table 2. Entropy values for Brij S10/Tween 60 system.

CONCLUSIONS: In Brij S10 – Tween 20 mixed micelle, due to the shorter hydrophobic segment of Tween 20 molecule, Brij's alkyl chain is more likely to assume a globular conformation in the micelle interior than it is the case in Brij S10 – Tween 60 system. For every analyzed system, with the rise in temperature, β parameter decreases, suggesting that the hydrophobic chains of studied surfactants are more likely to assume a globular conformation. Higher values of conformational freedom indicate that the process of micellization is followed by an increase in entropy. Gibbs free energy of micellization is, therefore, defined by entropic contributions as well as enthalpic input.

MAGNETITE BASED SAMPLES FOR MAGNETIC HYPERTHERMIA APPLICATIONS

Nina Novaković¹, Miloš Ognjanović²

¹ University of Novi Sad, Faculty of Sciences, Department of Physics,

² University of Belgrade, Institute of Nuclear Sciences "Vinca", Belgrade

*Corresponding author: ninan13@yahoo.com

KEYWORDS: Magnetic hyperthermia; Zn substituted Fe_3O_4 ; Microstructure; Nanoparticles

INTRODUCTION: Magnetic hyperthermia is a therapeutic procedure in which tissue is heated above its normal physiological temperature using magnetic nanomaterials in the alternative magnetic field. This approach involves the local increase in temperature of the tumor, where there is a change in the physiology of tumor cells, and ultimately this leads to their disappearance. This form of treatment is currently in research phase and it complements the currently available methods of treatment such as chemotherapy, radiation, surgery.

OBJECTIVES: Our main goal was to analyze influence of substitution of Fe^{2+} ions by Zn^{2+} in Fe_3O_4 on structural properties and heating ability. First objective was preparation of single phase spinel samples with different Zn concentration.

METHOD / DESIGN: Nanoparticles $\text{Zn}_x\text{Fe}_{3-x}\text{O}_4$ were synthesized by MW assisted hydrothermal synthesis route. Composition of the sample was analyzed by using X-ray diffraction (XRD). Size of particles and their distribution were examined using transmission electron microscope (TEM). Particles were coated with citric acid in order to prevent precipitation and to form stable colloids. Finally, heating efficiency (Specific Absorption Rate, SAR) of different samples in alternative magnetic field were measured using commercial DM1 device (nB Nanoscale, Spain).

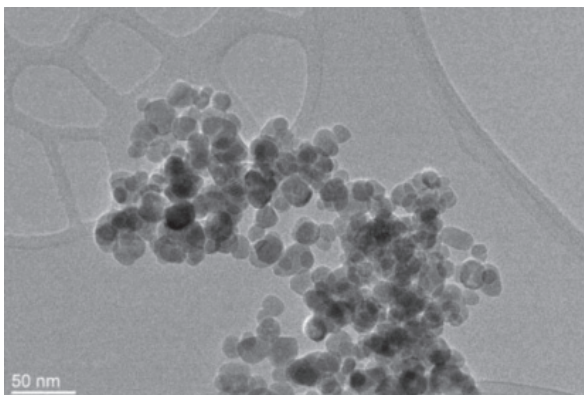


Figure1: TEM image of $\text{Zn}_{0.1}\text{Fe}_{2.9}\text{O}_4$

RESULTS: Using two-step method, co-precipitation accompanied by hydrothermal treatment in a microwave field, the samples $Zn_xFe_{3-x}O_4$ ($x=0.00-1.00$) were synthesized. X-ray diffraction pattern was indexed in the spinel structure type. Collected X-ray diffraction data were used to refine crystal structure by the Rietveld method. An analysis of line broadening (Scherrer equation) shown that crystallite size was decreased with increasing Zn concentration, in the range, 20-2.5 nm. TEM analysis (Figure 1) revealed that particles were pseudo-spherical. From results of SAR measurements it was concluded that samples $Zn_xFe_{3-x}O_4$ ($x=0, 0.1, 0.2$) with more percent of Zn atoms had better heating efficiency. The found values of SAR were in the range 90.97-635.27 W/g.

CONCLUSIONS: The samples $Zn_xFe_{3-x}O_4$ were prepared in a two step procedure, co-precipitation folowed by hydrothermal treatment in microwave field. Influence of Zn concentration in serie $Zn_xFe_{3-x}O_4$ was shown. By partial cation substitution it is possible to tailor physical/chemical properties of nanosize magnetite. Results on physical and chemical investigations point to that $Zn_xFe_{3-x}O_4$ /citric acid nanoparticles from this study could be further considered for Magnetic hyperthermia applications in cancer therapy.

MICROENCAPSULATION OF CHOKEBERRY POLYPHENOLS AND ANTHOCYANINS WITH DIFFERENT CARRIER BY SPRAY DRYING METHOD

Nada Čujić¹, Katarina Šavikin¹, Teodora Janković¹, Ana Kalužević², Viktor Nedović², Svetlana Ibrić³

¹ *Institute for Medicinal Plants Research, T. Košćuška 1, 11000 Belgrade, Serbia*

² *Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade, Zemun, Serbia*

³ *University of Belgrade, Faculty of Pharmacy, Vojvode Stepe 450, 11000 Belgrade, Serbia*

*Corresponding author: ncujic@mocbilja.rs

KEYWORDS: polyphenols; anthocyanins; microencapsulation; chokeberry; spray drying

INTRODUCTION: Chokeberries, as one of the richest plant sources of phenolics, especially anthocyanins, play important role in human nutrition and the growing interest for their utilization is mainly due to their antioxidant potential. Problem with polyphenols instability can be solved by microencapsulation technology, which has tendency to improve the stability of extracts during storage and processing. Spray drying is simple, low cost and one of the most convenient microencapsulation method for controlled production of small particles with uniform and desirable size.

OBJECTIVES: Encapsulation of 50% chokeberry ethanolic extract-CE with different carriers was employed. Encapsulation systems were examined and compared in order to choose the optimal one.

METHOD / DESIGN: Spray drying method was applied for microencapsulation of 50% CE (2.52mg GAE/ml) (the ethanol was evaporated) under the below-stated spray-drying conditions. Three different biopolymers: arabic gum (5%, w/v), maltodextrin (20%, w/v) and summed milk (20%, w/v) were used for microencapsulation. A spray dryer (Büchi mini B-290, Büchi Labortechnik AG, Switzerland) with a 0.7 mm standard diameter nozzle, with inlet (130±3°C) and outlet (56±2°C) temperatures, was used. Relatively low spraying air flow rate (536 L/h), liquid feed (8 mL/min rate), atomization pressure (6 psi) and low inlet temperature were chosen in order to ensure a good atomization along the liquid feed rate and to obtained a stable powder (especially to ensure anthocyanins stability) with high actual loading. The release profiles for the polyphenols and anthocyanins from microbeads in water were investigated determining the total polyphenol content-TP using Folin-Ciocalteu method and total anthocyanins content-TA using the procedure described in European Pharmacopoeia 6.0. (2008) with slight modifications. Transmission spectra of obtained spray-dried powders were recorded using a IRAffinity-1 Fourier transform infrared spectrophotometer (Schimadzu, Japan) and applying the KBr disc method. Microbeads were mixed with potassium-bromide powder and compressed to pastilles. Infrared spectra over the wavelength range 4000 to 600 cm⁻¹ were recorded and the resolution was

4 cm⁻¹. The size of the encapsulated microbeads was determined using Mastersizer 2000 (Malvern Instruments, Worcester-shire, UK). The mean diameter over volume (also called DeBroukere mean) was used as representative diameter.

RESULTS: The matrix influence on the active compounds stability was evaluated. The impact of different type of carriers on phenolics and anthocyanins release has been observed. The best results of TP release were achieved with arabic gum as a carrier (2.168 mg GAE/g), than with summed milk (1.790 mg GAE/g) and maltodextrin (1.203 mg GAE/g). The same trend was achieved in the case of TA release with arabic gum, which showed the best results (0.040%), in comparison with maltodextrin (0.031%) and summed milk (0.022%). Phenolic and anthocyanins compounds were released very rapidly from spray dried microbeads. Particle size for obtained microparticles were 8.50 µm for skimmed milk, 14.92 µm for arabic gum and 15.87 µm for maltodextrin. Determining size of obtained particles confirmed that spray drying is suitable method for production of small particles with uniform size. Microbeads obtained with skimmed milk were smaller in comparison with arabic gum and maltodextrin powders and desirable particle size depends on future application. Larger particles provide more extended release of encapsulated compounds, while smaller beads have better organoleptic characteristics, which is important for application in food products. FTIR spectrum of encapsulated CE in maltodextrin, skimmed milk and arabic gum showed several relevant peaks. Peaks in range about 1000 cm⁻¹ could be attributed to the -C-O of cyclic ether and alcoholic groups from polyphenols, especially expressed with maltodextrin. FTIR analysis showed that CE was successfully incorporated into the particles, probably by intermolecular interactions and it could be concluded that used matrices are compatible materials for extract encapsulation.

CONCLUSIONS: Our result showed that stability of polyphenols, especially anthocyanins might be improved using spray drying as microencapsulation technology. Chokeberry polyphenol microbeads, due to their antioxidant potential, represent a promising food additive for incorporation into dietary supplements or functional food.

CONVERSION OF FRUIT INDUSTRIAL PROCESSING WASTE TO ACTIVATED CARBON SORBENT AND ITS EFFICIENCY FOR ORGANIC AND INORGANIC MICROPOLLUTANTS ADSORPTION

Maja Turk Sekulić¹, Sabolč Pap¹, Tatjana Šolević Knudsen², Nikola Bošković¹, Maja Sremački¹, Olivera Paunović¹, Jelena Radonić¹

^a *Faculty of Technical Sciences, Department of Environmental Engineering and Occupational Safety and Health, University of Novi Sad, Trg Dositeja Obradovića 6, 21 000 Novi Sad, Serbia*

^b *Institute of Chemistry, Technology and Metallurgy, Department of Chemistry, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia*

*Corresponding author: majaturk@uns.ac.rs

KEYWORDS: chlorophenols; cadmium; low cost adsorption; micropollutants; organic waste

INTRODUCTION: The aim of this study was to produce the low cost activated carbon at optimum production conditions from the fruit industrial processing waste by thermochemical activation. The applications of this carbon are primarily focused on the removal of several organic and inorganic micropollutants from water and wastewaters. The term “micropollutants” means organic or mineral substances whose toxic, persistent and bioaccumulative properties may have a negative effect on the environment or organisms. They are present in many products that we consume daily, at the home or in industry. Some of these substances are liable to have potentially chronic direct or indirect effects on ecosystems, and even on human health. Major families of micropollutants are metals and metalloids, radioactive elements, organic micropollutants, hormones, pharmaceutical products and endocrine disruptors. The technologies employed for the treatment of micropollutants in water depend on the physico-chemical characteristics of each compound present in water. In the same family, many varieties of components can be found: hydrophilic or hydrophobic, adsorbable, volatile or semi-volatile, biodegradable, refractory, high or low molecular weight. The treatment processes used for their removal are biological degradation, adsorption, oxidation and the membrane separation technique. Activated carbon is one of the most commonly used adsorbents in the removal process of industrial pollutants. With the goal of diminishing the cost of producing activated carbon, contemporary research is taking a turn towards agricultural, industrial or vegetable (lignocellulosic) wastes to be used as raw material, and, then, lessen the cost of production. Besides, the use of these precursors reduces residue generation in both rural and urban areas. The synthesis and characterization of activated carbons obtained from lignocellulosic precursors is a topic widely studied by a number of researchers worldwide.

OBJECTIVES: The objectives of the research was focused on selection, development, characterization and evaluation of the efficiency of cost-effective adsorption medium

for the removal of chlorophenols (dichlorophenol, trichlorophenol, tetrachlorophenol, pentachlorophenol) and divalent cadmium cations from aqueous systems, as a novel, eco-friendly solution for wastewater remediation. The primary source of biomass for the production of low-cost adsorbent were lignocellulosic raw materials - apricot kernels, as industrial waste byproducts and components of organic solid waste.

METHOD / DESIGN: Activated carbon synthesis was carried out by thermochemical conversion, applying phosphoric acid at 500 °C, in the complete absence of nitrogen inert atmosphere. A detailed physico-chemical characterization of the obtained activated carbon was performed through various instrumental analyses, comprising of FTIR, SEM, and BET.

RESULTS: BET surface area evaluated using nitrogen adsorption isotherm corresponds to 1098,78 m² g⁻¹ (mesopore vol: 0,022 cm³ g⁻¹; mesopore sur. area: 16,87 m² g⁻¹; max mesopore diameter: 31,97nm; micropore volume: 0,391 cm³ g⁻¹). The maximum adsorption efficiency of the activated carbon for Cd²⁺ ions was 99%. The experimental results indicate that 20 mg of activated carbon removed max 87% of 500 ng L⁻¹ chlorophenols.

CONCLUSIONS: The present investigations qualified the apricot kernels as sustainable, low-cost, locally available and good waste materials for efficiency adsorption of chlorophenols and Cd²⁺ metal ions from aqueous wastes.

RIVERINE PLANT HABITATS DETERIORATION ASSESSMENT USING SERCON VS. REMOTE SENSING (AIRBORNE) INTERPRETED DATA

Milica Živković¹, Dušanka Cvijanović¹, Ana Anđelković^{1,2}, Danijela Pavlović,
Maja Novković¹, Branislav Vesković³, Snežana Radulović¹

¹ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

² Institute for Plant Protection and Environment, Belgrade, Serbia

³ Airworx, Novi Sad, Serbia

*Corresponding author: maja.novkovi@dbe.uns.ac.rs

KEYWORDS: Plants, Aquatic, RHS, alternation, remote sensing

The aim of this paper is to portray and analyze the hydromorphological characteristics of 25 rivers in Serbia (the Middle Danube Basin), with regards to the geographical characteristics of the basin area, by applying the RHS application in SERCON (System for Evaluating Rivers for Conservation) software and comparing it with remote sensed data. Surveying the morphology of river channels, the level of physical habitat degradation due to human activity has traditionally been carried out in the field. The weakness of such field surveys is that they are rather parochial in extent, over time consuming and too expensive. SERCON is a technique for the evaluation of a large number of characteristics related to the habitats, hydrobionts and the entire river basin area, including all potential influences of the degradation of its natural state and the collection of the information pertaining to the characteristics which could be used with the aim to better represent a certain river by the accepted criteria of active preservation SERCON. Information on each attribute is used to create a picture of a river in terms of accepted conservation criteria such as Naturalness, Representativeness and Rarity. SERCON software was developed at the Faculty of Sciences in Novi Sad and implemented as a client-server application (<http://sercon.pmf.uns.ac.rs/SerconWeb>).

The field research was conducted during the summer months of 2010-2014 along 25 rivers in Serbia (the Middle Danube Basin). The RHS scores (HQA – *Habitat Quality Assessment* and HMS - *Habitat Modification Score*) were calculated in the SERCON software. The matrix of RHS scores was tested by applying the detrended correspondence analysis (DCA), while the canonical correspondence analysis (CCA) in the CANOCO 4.5 programme package was used for further analysis.

Application of the RHS method has enabled us to determine the hydromorphological characteristics of 25 rivers in Serbia. The most represented vegetation types which have been recorded are: the submerged fine-leaved type, with the dominant association of *Ceratophylletum demersi* Corillion 1975, the floating non-rooted type with the association *Lemno-Spirodeletum* W.Koch 1954 and the emergent reeds vegetation type with *Scirpo-Phragmitetum* Koch 1926 as the dominant association in this vegetation type. HQA values for individual RHS sections varied between 4 and 73, while the HMS score fluctuated between 0 and 6990. Negative correlation between the RHS scores

implies that hydromorphological pressures directly affect the habitat diversity degradation. CCA analysis has determined the significance of the basin area size, altitude and geological substrate, when compared with the variation of RHS scores and subscores. These variables have explained 7.72% of the RHS score variation.

Remotely sensed digital imagery provides a synoptic coverage of riverine systems and monitoring change, more effective in many ways and much less costly on a long-term basis.

In this study the results obtained by field assessment were compared with the data obtained by remote sensing techniques, which is more often used to monitor water quality parameters, such as suspended sediment, chlorophyll and temperature). The study demonstrates that high spatial resolution remotely sensed digital imagery has the potential to be a useful tool for panoptic mapping of the geomorphology and human impact on river systems. and estuaries. The possibilities and restraints were discussed.

EVALUATION OF THERAPEUTIC POTENTIAL OF RECOMBINANT H1D2 CHIMERA IN MODULATION OF THE SPECIFIC IMMUNE RESPONSE IN BALB/C MICE

Ivan Mrkić¹, Rajna Minić², Tanja Bulat³, Branko Drakulić⁴,
Marija Gavrović-Jankulović⁵

¹ *Innovation Center, Faculty of Chemistry University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

² *Institute of Virology, Vaccines and Sera, Torlak, Vojvode Stepe 458, 11221 Belgrade, Serbia,*

³ *University of Vienna, Faculty of Life Sciences, Althanstrasse 14, 1090 Vienna, Austria*

⁴ *Department of Chemistry-ICHtM, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia*

⁵ *Department of Biochemistry, Faculty of Chemistry University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia*

*Corresponding author: Ivan Mrkić: mrkicivan@chem.bg.ac.rs

KEYWORDS: allergen; Dust mite; influenza; hemagglutinin

Allergens from house dust mite, particularly Der p1 and Der p2 are major contributors to the respiratory allergic diseases, and are regarded as a target for allergen specific immunotherapy. A therapeutic potential of a chimera made of Der p 2 (D2) linked to Influenza A virus hemagglutinin 1 (H1) for intranasal application was designed, expressed and tested in a mouse model. H1D2 and D2 proteins were produced by genetic engineering in *E. coli* and their primary structure was confirmed by mass spectrometry. Produced recombinant proteins preserved IgE reactivity in immunoblot with serum from house dust mite allergic persons. Balb/c mice were sensitized with D2 allergen in alum and subsequently received H1D2 or D2, intranasally. Levels of D2 specific antibodies (IgE, total IgG, and IgA) were determined by ELISA. Reduced levels of serum specific IgE together with increased serum specific IgG and IgA levels were detected in both groups which received H1D2 and D2 intranasally. Higher level of CD4+CD25+ spleen lymphocytes was found in the group of mice which received i.n. H1D2 when compared to the group which received i.n. D2.

H1D2 chimera can have a therapeutic potential as dual vaccine particularly in Der p 2 allergic persons which beside allergen specific can provide protective antibodies against Influenza A virus hemagglutinin 1.

HUMIDITY SENSING WITH PAPER-BASED SENSORS COATED WITH ZNO NANOPARTICLES

Georgios Niarchos¹, Georges Dubourg¹, Georgios Afroudakis², Vasiliki Tsouti², Eleni Makarona², Jovan Matovic¹, Vesna Crnojevic-Bengin¹, Christos Tsamis²

¹ BioSense Institute, Novi Sad, Serbia

² Institute of Nanoscience and Nanotechnology, National Center for Scientific Research "Demokritos", Patriarhou Gregoriou & Neapoleos, Aghia Paraskevi, Athens, Greece

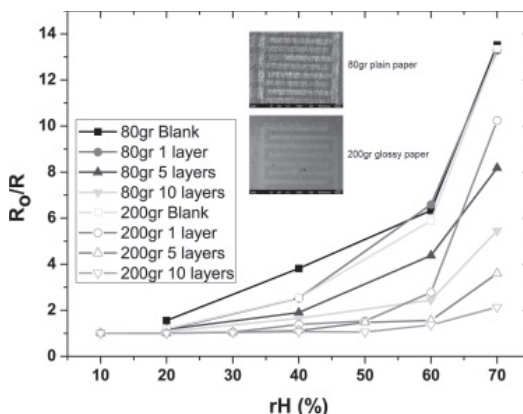
*Corresponding author: niarchos@uns.ac.rs

KEYWORDS: Humidity sensor; paper substrate; ZnO nanoparticles; laser micromachining;

INTRODUCTION: Paper, has recently emerged as a promising material for flexible and lightweight devices, mainly due to its low-cost, flexibility and potential for mass-production of disposable devices. Paper in essence consists of wood cellulose fibers and offers a wide range of desirable properties, such as biodegradability, chemical compatibility with various substances and ease-of-fabrication, making it a perfect candidate for cost-efficient and easy-to-implement sensing applications.

OBJECTIVES: In this work, we investigate the performance of humidity sensors fabricated on simple, commercial printing paper substrates and the influence of ZnO nanoparticles on their operating behavior.

METHOD / DESIGN: Humidity sensors were fabricated on two different paper substrates, one with 80grm⁻² basis weight and a glossy with 200grm⁻² basis weight, using a combination of standard deposition techniques and laser micromachining. A thin Au film was sputtered directly on the paper substrates and then, using a short pulse laser the Au layer was ablated and interdigitated electrodes (IDEs) were patterned. Sol-gel solutions containing ZnO nanoparticles were spin-coated on the IDEs and annealed at low temperature (100°C) to enable the formation of a compact film.



RESULTS: Resistive measurements across the IDEs, for known relative humidity (rH) levels, were performed under room temperature conditions to assess the sensing properties of the sensors as a function of the sol-gel composition and the number of coating layers. Results indicate that the ZnO film passivates the paper substrate towards humidity and this behavior depends on the total number of deposited films on the sensor surface.

CONCLUSIONS: The sensing mechanism and operating principles of humidity paper-based sensors coated with ZnO nanoparticles have been investigated and evaluated. Sensors are able to operate at room temperature without refreshing after each measurement. Coatings of ZnO nanostructured film offer a passivation layer towards humidity, directly related to the number of layers. These results pave the way to the fabrication of low-cost and impervious to humidity devices for sensing applications.

THE ASSESSMENT OF COMMON REED EXPANSION (*PHRAGMITES AUSTRALIS* (CAV.) TRIN. EX STEUD.) ON THE LUDAŠ LAKE USING SATELITE IMAGINERY

Jasna Grabić,¹ Pavel Benka¹, **Vladimir Ćirić**¹, Predrag Lugonja², Nenad Antonić¹

¹ University of Novi Sad, Faculty of Agriculture, ²University of Novi Sad, Institute BioSense

*Corresponding author: vladimir.ciric@polj.uns.ac.rs

KEYWORDS: common reed, eutrophication, remote sensing, GIS

INTRODUCTION: The Ludaš Lake is situated in the north part of the Autonomous Province of Vojvodina close the border with Hungary. This shallow saline lake originates from draining of sandy area and a series of salty depressions and from the River Kireš. The lake banks are overgrown with common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) representing a nesting place for a numerous bird species. All these features contributing to its uniqueness and as a center of biodiversity led to its listing as Ramsar site and a part of the Important Bird Area. Beside its international significance, Serbian regulation has pronounced it as a special nature reserve. However, despite the natural beauty of the lake, it has been seriously polluted due to anthropogenic influences. The lake has been surrounded by leaching of surplus nutrients from surrounding arable land, municipal diffuse pollution from nearby settlements and from canal which conducts water from also polluted Lake Palić. All sources of pollution are contributing to intensification of eutrophic processes frequently observed as algal blooms, but also facilitating spreading of common reed. While the phenomenon of algal blooms has been investigated, expansion of common reed has not been quantified yet.

OBJECTIVES: The aim of this research was to make comparison of areas covered by common reed in 2001 and in 2016. In order to achieve the overall goal, objectives were to identify total lake area, and then to distinguish water table from areas covered by common reed, for both investigated years.

METHOD / DESIGN: Spatial data were acquired from the Google Earth program, from which 2 suitable satellite images were taken for 2001 and 2016. Baring in mind different condition within the lake, its overall area was divided into 3 sections: north part, middle part and south part. Applying geographic information system (GIS) software - QGIS, Essen 2.14, borders between water and common reed were digitalized on the images. Upon identified borders polygons were formed – representing areas under common reed and water. Finally, perimeters and areas under common reed and water were calculated for each section.

RESULTS: The results showed an increase of areas under common reed over the Lake Ludaš during the investigated period 2001-2016. The overall increase was 20.35 ha (15.93%) whereas for north, middle and south section these were: 12.25 ha (15.59%), 5.95 ha (37.11%) and 2.17 (6.57%) respectively. Besides, perimeter of common reed

areas borders showed decrease for 4.32 km (4.01%). This indicates that small areas covered by the common reed were grouped into larger ones, except for the middle section where an increase was observed (Table 1).

Table1. Spreading of common reed over the Lake Ludaš in period 2001-2016.

Year/ period		Units	Total for Lake Ludaš	North section	Middle section	South section
2001	Total area	ha	341.99	202.08	75.71	64.20
	Common reed	ha	127.71	78.61	16.04	33.07
	Common reed border	km	107.75	70.86	20.60	16.86
2016	Total area	ha	338.46	200.46	74.69	63.31
	Common reed	ha	148.06	90.86	21.99	35.25
	Common reed border	km	103.43	65.57	22.59	15.92
Difference for 2001 - 2016	Common reed	ha	20.35	12.25	5.95	2.17
	Common reed	%	15.93	15.59	37.11	6.57
	Common reed border	km	-4,01	-7,46	9,67	-5,52

CONCLUSIONS: Common reed is a dominant macrophyte on the Lake Ludaš which enables nesting place for a variety of bird species. Although all shallow lakes are subject to natural succession processes leading to formation of terrestrial ecosystems, negative anthropogenic influences may speed up the eutrophication processes. Such an example is the Lake Ludaš where, according to the results of this investigation area under common reed has increased for 20.35 ha (15.93%) in period 2001-2016. In order to give exact future estimations about the trend of common reed spreading further investigation is needed.

RENEWABLE ENERGY SCENARIO: CURRENT STATUS AND PROSPECT OF BANGLADESH

Nasir Uddin¹, Kuaanan Techato¹, NA Nithe¹, Ahasan Habib¹, JI Rony²

¹ Faculty of Environmental Management, Department of Sustainable Energy Management, Prince of Songkla University (PSU), Hat-Yai, Songkhla, Thailand,

² Department of Electronics Engineering, Bangladesh Technical Education Board (BTEB), Bangladesh

*Corresponding author: engnasirbd@gmail.com

KEYWORDS: Renewable Energy; Solar; Electricity; Bangladesh; Fossil fuel.

INTRODUCTION: Electricity is an unavoidable prerequisite developmental resource for an advancement of the nation. Today in Bangladesh share percentage of renewable energy only 3% of total energy ratio. Bangladesh has already taken a master plan in renewable energy sector.

OBJECTIVES: The current renewable energy agenda of Bangladeshi government force the specialization of renewable energy generation budget by decreasing global pollution with save movement of biomass, solar, hydro, wind, and tidal power sector.

METHOD / DESIGN: This paper presents the currents national energy scenario of Bangladesh. According to this, the grater potentiality of renewable energy resources is also reviewed and presented in this paper.

RESULTS: In Bangladesh, the proved reserved of natural gas 34 TCF which will lead the country next 20 years and currently 82% natural gas consumed in the power sector for power production. Only 3% of electricity is produced from renewable energy sources.

CONCLUSIONS: Bangladesh government have already announced a master plan for future electricity generation though the demand of power growing faster rate. This strategy emphasizes the countries natural resource exploration and discoveries for further improvement of power sector and reconstruct the renewables energy resource. However to meet the near future demand Bangladesh needs more sophisticated research facilities and skilled manpower for exploration activities both in land and off-shore areas. The government must work with international advanced technology using human intellectuals of the country for energy sustainability.

TWO STROKE AIR COMPRESSED MACHINE FROM IC ENGINE

Nasir Uddin¹, Kuaanan Techato¹, NA Nithe¹, Ahasan Habib¹, JI Rony²

¹ Faculty of Environmental Management, Department of Sustainable Energy Management, Prince of Songkla University (PSU), Hat-Yai, Songkhla, Thailand,

² Department of Electronics Engineering, Bangladesh Technical Education Board (BTEB), Bangladesh

*Corresponding author: engnasirbd@gmail.com

KEYWORDS: compressed air; compressed machine; power output.

INTRODUCTION: The world is going on energy crisis, available fossil fuel reserves are in critical condition and environment compounded by pollution of all kinds, while any technologies that bring out the solutions to this problem is considered as a bounty. Among of the solutions of this problem, compressed air driven machine technology is one possibility. This solution does not require any types of fossil fuels and is driven by compressed air as a fuel source.

OBJECTIVES: The main objective is to design, modify and run the petrol engine by compressed air, without any fuel. For this purpose some special change in engine construction was done by camshaft lobe modification.

METHOD / DESIGN: A general four stroke petrol engine (IC engine) has been converted into two stroke air compressed machine. The power output is being examined by supplying compressed air from the air compressor. This method will demonstrate the concept of green environmentally friendly new engine technology for the future.

RESULTS: The experimental results show a promising maximum efficiency percentage of 53.42, 35.6, 30.4, 26.67 and 23.60 under the pressure of 2 to 4.5 bar with the maximum load condition.

CONCLUSIONS: In the field of automobile technology it is a revolutionary dimension for the future design of hybrid fuel air engine. There are few automobiles designed as air car for future generations, like in France (mdi international), India (Tata motors) and Australia (engine depiero).

In this experiment, the operation of tested engine was driven with compressed air engine demonstrated the feasibility of vehicle applications. However, some lacking's also had showed during experiment with lower pressure compressor air supply. Further applications on motor vehicles will need to take the advantages of air compressed machine operation and integrate with conventional IC engine.



ABSTRACTS

Track 4: Bioactive natural products – Biochemistry and Pharmacology

T4





Wilawan Mahabusarakam

GARCINIA DULCIS KURZ: SECONDARY METABOLITES AND BIOLOGICAL ACTIVITY

Wilawan Mahabusarakam^{1,3}, Suwanna Deachathai¹, Parichat Thepthong¹, Arun Saelee¹, Pattama Mecawun¹, Ilham Abdullah¹, Souwalak Phongpaichit^{2,3}

¹ Department of Chemistry, ²Department of Microbiology,

³Natural Product Research Center of Excellence, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

*Corresponding author: wilawan.m@psu.ac.th

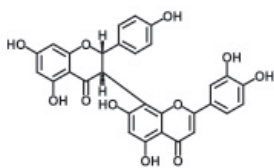
KEYWORDS: Clusiaceae; *Garcinia dulcis*; xanthonenes; flavonoids; biological activity

INTRODUCTION: *Garcinia dulcis* (Roxb.) Kurz. (Clusiaceae) is widely distributed in Thailand and other Southeast Asian countries. It has been used in traditional medicine for centuries. In Indonesia, the leaves and seeds have been used for the treatment of lymphatitis, parotitis and struma. In Thailand, its stem bark has been used as an anti-inflammatory agent. The fruit juice has been used in traditional medicine as an expectorant while the root extract as an antipyretic and antitoxin agent

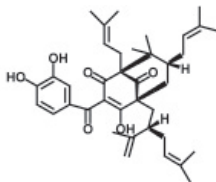
OBJECTIVES: The results from preliminary screening on biological activity of crude extracts from *G. dulcis* collected in Thailand, together with the knowledge that other members of the genus *Garcinia* are known to be a source of bioactive compounds led us to examine this plant species for substances active for antioxidation and as antibacterial agents.

METHOD / DESIGN: The fruits, seeds, flowers, branches, leaves, roots, and stem barks of *G. dulcis* were extracted with organic solvent such as methylene chloride, acetone and methanol. The crude extracts were purified using column chromatography and PTLC. The structures of the isolated compounds were determined by analysis of 1D and 2D NMR spectroscopic data. The activities on antibacterial and antioxidative of the crude extracts and pure compounds were examined.

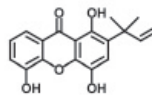
RESULTS: Twenty four new xanthonenes, three new biflavonoids, and ninety-three known compounds were isolated from *G. dulcis* collected in Thailand. Sixty seven distinct xanthonenes have been identified in different parts of the plant, with thirty-six being present in the stem bark. Morelloflavone, a biflavonoid, was a major constituent in leaves, flowers, branches, and root barks. Camboginol was the most abundant



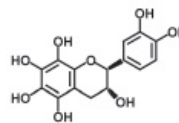
Morelloflavone



Camboginol



12b-Hydroxy-des-D-garcigerrin A



Dulcisflavan

compound in the fruits. 12b-Hydroxy-des-D-garcigerrin A showed strong antibacterial activity with MIC 2 g/mL. Dulcisflavan acted as a strong antioxidant with IC_{50} 5.9 M.

CONCLUSIONS: *Garcinia dulcis* Kurz is an abundant source of phenolic compounds including xanthenes, biflavonoids, prenylated benzophenones, flavonoids. Some of the compounds exhibited very effective antibacterial and antioxidative activity.



Olga Tzakou

ESSENTIAL OILS AS GREEN PESTICIDES

Olga Tzakou¹

¹ *Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zographou, 157 71 Athens, Greece*

**Corresponding author: tzakou@pharm.uoa.gr*

Essential oils find a huge application area in medicine and aromatherapy in view of the fact that they have a wide spectrum of diverse impressive biological activities. Plant essential oils have been recognized as an important natural source of pesticides. The essential oils are relatively safer and pose fewer risks to the environment compared to the synthetic insecticides, due to their rapid environmental degradation, with minimal impacts to animal and human health. In recent times there is an urgent need for controlling of insects due to the arising problem of insect transmitted pathogens such as Zika fever, West Nile disease, malaria, yellow fever, which affect millions of people annually worldwide. Essential oils are distributed in a limited number of families and their biopesticidal potential indicates that plant families such as Myrtaceae, Labiatae, Compositae, Umbelliferae and Rutaceae exhibit significant activities against specific orders of insects like Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera. Essential oils have been explored for repellent, fumigant, larvicidal and adulticidal activities against insects. Currently essential oil-based commercial products are being developed for a wide range of human and animal uses, including insect control.



Juraithip Wungsintaweekul

**CYCLOOXYGENASES AND INDUCIBLE NITRIC OXIDE SYNTHASE
INHIBITORY ACTIVITIES OF DITERPENES FROM *CROTON
STELLATOPILOSUS***

Juraithip Wungsintaweekul^{1,2}, Charoenwong Premprasert¹, Supinya Tewtrakul^{1,2}

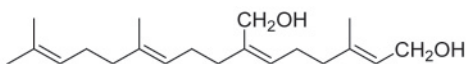
¹ Department of Pharmacognosy and Pharmaceutical Botany,

² Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

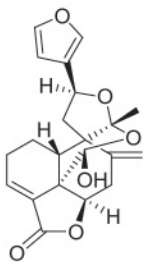
*Corresponding author: juraithip.w@psu.ac.th

KEYWORDS: *Croton stellatopilosus*; plaunotol; plaunol A; plaunol E; plaunol F; RAW264.7 cells

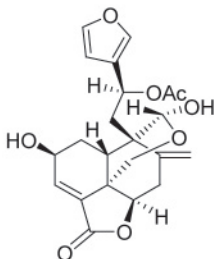
INTRODUCTION: Plaunoi [*Croton stellatopilosus* Ohba; Euphorbiaceae] is a woody plant and indigenous to Thailand. Plaunoi was formerly well known as *C. sublyratus* Kurz and used traditionally in combination with Plauyai (*C. oblongifolius* Roxb.) for widely purposes such as anthelmintic, digestant, tranquilizer, stomachic and used as a dermatologic agent for skin diseases. Plaunotol, a principle component, was registered and prescribed for cytoprotective agent (The Sankyo Ltd., Tokyo, Japan).



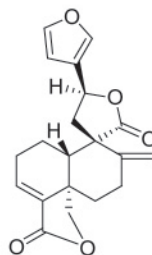
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OBJECTIVES: The present study aimed to evaluate the cyclooxygenases (*COX-1* and *COX-2*) and inducible nitric oxide synthase (*iNOS*) inhibitory activities of diterpenes isolated from *C. stellatopilosus* leaves.

METHOD / DESIGN: Diterpenes were isolated from *C. stellatopilosus* leaves, guided by determination of the inhibitory activity on nitric oxide (NO) production in the macrophage RAW264.7 cells. Quantitative RT-PCR was used as a tool for determination of mRNA levels of *COX-1*, *COX-2* and *iNOS* genes.

RESULTS: Plaunotol [1] and three furanoditerpenes-plaunol A [2], plaunol E [3] and plaunol F [4] were assessed for their inhibitory activity on NO production by LPS-induced RAW264.7 cells. They exhibited inhibitory activity with IC₅₀ values of 3.41, 12.10, 17.09 and 2.79 μ M, respectively. Transcription profiles of the *COX-1*, *COX-2* and *iNOS* genes were measured by qRT-PCR. The results indicated that all diterpenes suppressed *iNOS*, on the other hand, plaunotol stimulated *COX-1* and *COX-2* genes. Moreover, three furanoditerpenes downregulated the *COX-1* and *COX-2* genes.

CONCLUSIONS: In summary, this study shows that the diterpenes from *C. stellatopilosus* exhibit an anti-inflammatory activity towards LPS-induced RAW264.7 cells by different mechanisms. Our results provided data to support further investigations into the possibility that these diterpenes could be alternatives to act as anti-inflammatory agents.



Nongyao Sawangjaroen

COPTOSAPELTA FLAVESCENS KORTH. AS A POTENTIAL ANTI-GIARDIAL COMPOUNDS SOURCE

Nongyao Sawangjaroen¹, Kruawan Hounkong¹, Wipapan Kongyen², Vatcharin Rukachaisirikul³

¹ Department of Microbiology, Faculty of Science, and Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand,

² Chemistry and Applied Chemistry Program, Faculty of Science and Technology, Songkhla Rajabhat University, Mueang, Songkhla 90000, Thailand,

³ Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

*Corresponding author: nongyao.s@psu.ac.th

KEYWORDS: anti-protozoan; diarrhea; *Giardia intestinalis*; medicinal plant; *Coptosapelta flavescens*

INTRODUCTION: *Giardia intestinalis* is a most common pathogenic intestinal protozoan parasite. It is a major cause of traveler's diarrhea where tap water is the primary source of drinking water. Metronidazole, the most commonly used anti-protozoan, caused unpleasant side effects such as a metallic taste, headache, nausea, urticaria, pruritus and dark colored urine.

OBJECTIVES: To search for a new anti-protozoan compound with high activity that is safe and produces little or no side effects from Thai medicinal plants which represent a rich source of potential antiparasitic compounds.

METHOD / DESIGN: Several extracts from Thai medicinal plants which were claimed to possess anti-diarrhoeal or anti-parasitic activity, were screened for their activity against *G. intestinalis* trophozoites *in vitro*. The most active extract was then further purified to obtain the most potent pure compound. Its mechanisms of actions against *G. intestinalis* trophozoites were then evaluated *in vitro*, by Scanning electron micrographs (SEM) and TUNEL assays.

RESULTS: 1-hydroxy-2-hydroxymethylanthraquinone (CFQ), purified by column chromatography from *Coptosapelta flavescens*, the plant that commonly used to expel intestinal worm, had shown to possess the most potent activity. This was comparable to the standard drug, metronidazole. At their IC₅₀ value (0.42 µg/mL) both metronidazole and CFQ caused several physical alterations to *G. intestinalis* trophozoites when view

under SEM (Fig 1). CFQ also induced apoptosis as early as 6 h incubation, whereas metronidazole produced little or no apoptosis. At 24 h incubation, CFQ caused 74.8% of *G. intestinalis* trophozoite to undergo late apoptotic stage. This effect was confirmed by TUNEL assay that chromatin in most of *G. intestinalis* trophozoite exposed to CFQ was condensed, fragmented and stain orange colour. Cell cycle analysis showed that the distribution of *G. intestinalis* DNA treated with metronidazole and CFQ were not significantly different from the control. It is indicated that both drugs had no effect on *G. intestinalis* cell cycle. On the other hand, both metronidazole and CFQ at IC_{50} concentrations caused a significant detachment of *G. intestinalis* trophozoites from Caco-2 cell line when compared to the control at 6 h incubation.

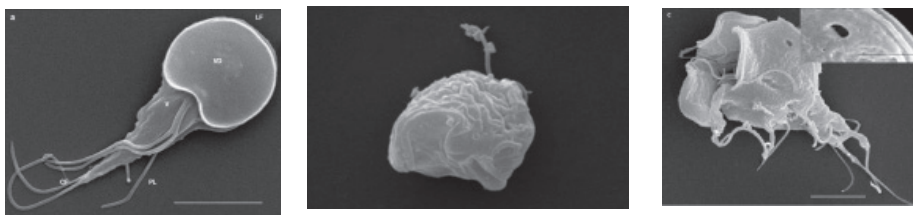


Fig. 1. Scanning electron micrographs of *G. intestinalis* trophozoites after being incubated at 37°C for 24 h in: a) complete medium; b) 0.42 µg/mL metronidazole, c) 0.42 µg/mL 1-hydroxy-2-hydroxymethylanthraquinone. Bars = 5 µm; insert figure, bar = 2 µm. CF = caudal flagella, LF = lateral flange, PL = posterior-lateral flagella, V = ventral flagella and VD = ventral disk

CONCLUSIONS: We have provided a mechanistic explanation for the action of CFQ against *G. intestinalis* trophozoites. These results have provided evidences that CFQ could be potentially a new compound for use against *G. intestinalis*.



DEVELOPMENT OF A NEW FORCE FIELD FOR MOLECULAR DYNAMICS SIMULATIONS OF DNA MOLECULES

Ivan Ivani^{1,2}, Pablo D. Dans^{1,2}, Agnes Noy³, Alberto Pérez⁴, Ignacio Faustino^{1,2}, Adam Hospital^{1,2}, Jürgen Walther^{1,2}, Pau Andrio^{2,5}, Ramon Goñi^{2,5}, Alexandra Balaceanu^{1,2}, Guillem Portella^{1,2,6}, Federica Battistini^{1,2}, Josep Lluís Gelpi^{2,7}, Carlos González⁸, Michele Vendruscolo⁶, Charles A. Laughton⁹, Sarah A. Harris³, David A. Case¹⁰ and Modesto Orozco^{1,2,7*}

¹ *Institute for Research in Biomedicine (IRB Barcelona), Baldiri Reixac 10-12, 08028 Barcelona, Spain,*

² *Joint BSC-IRB Research Program in Computational Biology, Baldiri Reixac 10-12, 08028 Barcelona, Spain,*

³ *School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, UK,*

⁴ *Laufer Center for Physical and Quantitative Biology, Stony Brook University, USA,*

⁵ *Barcelona Supercomputing Center, Jordi Girona 29, 08034 Barcelona, Spain,*

⁶ *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK,*

⁷ *Department of Biochemistry and Molecular Biology, University of Barcelona, 08028 Barcelona, Spain,*

⁸ *Instituto de Química Física “Rocasolano”, CSIC, Serrano 119, 28006 Madrid, Spain,*

⁹ *School of Pharmacy and Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK,*

¹⁰ *Dept. of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08540 USA.*

*Corresponding author: ivan.ivani@irbbarcelona.org

KEYWORDS: Molecular dynamics, Simulations, DNA, Protein, Force-fields

Classical force-fields are at the core of classical simulations, particularly of molecular dynamics (MD), a technique that is changing our view of the structure, flexibility and function of biological macromolecules. The central idea behind force-field development is to define a basic energy function and refine associated parameters by fitting model simulation results to experimental data, or quantum mechanical level calculations. Force-field development is tightly connected to the extension of simulation time scales. As molecular dynamics (MD) trajectories are extended, errors previously undetected in short simulations emerge, creating the need to refine the force-fields. We present parmbsc1, a new force-field for DNA atomistic simulations, which has been parameterized from high-level quantum mechanical data and tested for nearly 100 systems (125 μ s) covering most of the DNA structural space. Parmbsc1 provides results of high quality in diverse systems, solving problems of previous force-fields [1]. Furthermore, it has been assayed for more than 1 year in a set of β -testers, which confirmed the lack of artifacts and the improvement reached with respect to previous force-fields. The results obtained are of unprecedented quality, and open up new frontiers for the simulation of DNA. Parmbsc1 aims to be a reference force-field for

the study of DNA in the next decade. To manage and distribute parmbsc1 trajectory data and analysis, we have developed a new database framework that can be accessed through a web portal at <http://mmb.irbbarcelona.org/ParmBSC1/>. This work has been recently published in the journal *Nature Methods* [2].

- [1] Pérez, Alberto, et al. "Refinement of the AMBER force field for nucleic acids: improving the description of α/γ conformers." *Biophysical journal* 92.11 (2007): 3817-3829.
- [2] Ivani, Ivan, et al. "Parmbsc1: a refined force field for DNA simulations." *Nature methods* 13.1 (2016): 55-58.

IMMUNITY GENES AND ANTIMICROBIAL PROTEINS IN COLLEMBOLA, *FOLSOMIA CANDIDA*

Khrongkhwon Thammatinna¹, Patamarerk Engsontia¹

¹ *Molecular Ecology and Evolution Research Unit, Department of Biology, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand*

*Corresponding author: patamarerk.e@psu.ac.th

KEYWORDS: collembola; *Folsomia candida*; immunity genes; antimicrobial proteins

INTRODUCTION: Collembola is a diverse group of small wingless hexapod living mainly in soil habitats. Given this fact, they have been exposed to various pathogens in soil such as bacteria, fungi and viruses. For this reason, collembola may have adaptation in their immunity genes that are essential for survival. Immunity genes include genes involved in the recognition of pathogens, genes encoding proteins in signaling pathways, and genes encoding defense proteins, such as lysozymes and antimicrobial proteins (AMPs). AMPs are particularly interesting because of their potential applications in pharmacy (i.e. developing new drugs). The genetic basis of defense mechanism in collembola, however, is largely unexplored. Valuable AMPs from this lineage of animal await discovery. Recent advance in the next-generation sequencing technology makes it possible to identify the entire sets of interesting genes in non-model organisms. This study used *Folsomia candida*, an important organism for toxicology research, for the investigation.

OBJECTIVES: To identify immunity related genes and antimicrobial proteins in collembola, *Folsomia candida*, using transcriptome analysis.

METHOD / DESIGN: To construct a *F. candida* transcriptome, RNA were extracted from the whole body, converted to cDNA and sequenced using Illumina HiSeq 2000 platform. Paired-end reads (100 bp) were assembled into 138,315 contigs using Trinity, and the transcriptome database was made on a PC using BLAST+ 2.2.31. We performed local BLAST search (tBLASTn; *E* value = 100) on this database using immunity related genes and AMP genes from other insects as queries. To verify *F. candida* immunity related genes, we performed tBLASTn search against NCBI insect nucleotide collections using candidate *F. candida* genes as queries. Sequences which do not show significant similarity to other insect immunity genes were removed from a final gene list. To verify *F. candida* AMPs, we used AMP prediction tools of CAMPR3 (Collection of Antimicrobial Peptides database). Only proteins that were predicted as AMPs from at least 3 out of 4 methods were kept as putative *F. candida* AMPs. Protein classes of *F. candida* AMPs were assigned using BLAST tool of CAMPR3.

RESULTS: For recognition genes, we identified 5 peptidoglycan receptor proteins (PGRPs) and 8 gram-negative binding proteins (GNBPs). For the signaling genes, we

identified 14 Tolls, 1 myeloid differentiation primary response gene (MyD88), 2 pelles (Pll), 2 immunodeficiencies (IMDs), 2 Relishes (Rels), 9 JNK pathways (JNKs), 1 Janus kinases (JAKs) and 9 signal-transducer and activator of transcriptions (STATs). For the response genes, we identified 13 lysozymes (Lyss), 19 chitinase (Chts) and 12 prophenoloxidases (PPOs). We also identified 21 candidate AMPs from *F. candida* which can be assigned to 9 classes: Thaumatin, Penaeidins, Defensins, Cathelicidins, Bacteriocin, Hemiptericin, Attacin, Coleoptericin, and Small cytokines.

CONCLUSIONS: *F. candida* has all major immunity genes previously reported in other insects. However, the number of genes varies among species, e.g. there is an expansion of chitinase genes in *F. candida*. We reported 21 new collembola putative AMPs. This body of knowledge allows interested researchers to further investigate AMPs in other collembolan species and to further study the function of AMPs from collembola.

MULTI-LEVEL STRATEGY FOR ANALYSIS OF BIOACTIVE DRUG CONFORMATIONS

Sanja Živanovic^{1,2}, Adam Hospital^{1,2}, Modesto Orozco^{1,2,3}

¹ *Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain;*

² *Joint BSC-IRB Research, Barcelona, Spain;*

³ *Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain*

*Corresponding author: sanja.zivanovic@irbbarcelona.org

KEYWORDS: drug design; computational chemistry; pharmacology; development

INTRODUCTION: The procedure of drug discovery is very time and resources consuming process. Computer-aided drug design (CADD) is one of the powerful tools which can be used to increase the efficiency of the drug discovery. Estimating the relative free energy of a ligand in its target-bound state (i.e. the bioactive conformation) is necessary to optimize the potency of bioactive molecules and to improve the accuracy of SDBB methods.

OBJECTIVES: Our aim is to develop an efficient framework for finding the bioactive conformation of the flexible ligands. Since the bioactive conformation of the ligand may differ from the global minimum of the free ligand in the physiological environment, one has to evaluate the energetic cost required for adopting the bioactive conformation. A set of 100 crystal structures of pharmaceutically relevant drug-like molecules was tested using multi-level approach. We combined low-level method (LL) for sampling the conformational minima and high-level (HL) ab-initio calculations for estimating their relative stability.

METHOD / DESIGN: The method was automated and tested on various ligands with different numbers of atoms, charge and rotatable bonds. The analysis show that is necessary to perform Hamiltonian Replica Exchange simulations in order to explore all possible states of energy landscape of given dihedrals. Our findings suggest that the method is an effective way to improve analysis of the bioactive conformations of drug-like.

RESULTS: It is worth noting that present framework for multilevel strategy is a complex and long-term task, which requires a lot of rehearsals and implementations. Taking into account the flexible nature of molecules, protonation state and tautomeric forms, make our task even more challenging.

CONCLUSIONS: The proposed strategy may represent an efficient tool for predicting the conformational landscape of drugs while keeping a reasonable balance between chemical accuracy and computational cost.

SYNTHESIS OF 3-OXO BILE ACIDS: SELECTED PRODUCTS IN THE BIOMEDICAL RESEARCH AND THEIR STUDY BY FT-IR SPECTROSCOPY

Tanja Sarenac¹, Momir Mikov¹

1 University of Novi Sad, Faculty of Medicine, Department of Pharmacology, Toxicology and Clinical Pharmacology, 21000 Novi Sad, Hajduk Veljkova 3

**Corresponding author: momir.mikov@otago.ac.nz*

KEYWORDS: 3-oxo bile acids; FT-IR spectroscopy

INTRODUCTION: Study of 3-oxo of bile acids derivatives by infrared spectroscopy integrated with Fourier transformation (FT-IR spectroscopy) is used to obtain information about the interactions between the solute and solvent.

OBJECTIVES: The main objective is to examine and to provide better insight into the possibility of creating intermolecular interactions of bile acids in tests of associations and only-associations of bile acids and their use for medical purposes.

METHOD / DESIGN: Synthesis was performed a 12 α -hydroxy-3-oxo-5 β -cholanoic acid in 4 synthetic stages from deoxycholic acid. We have investigated the potential interactions of the carbonyl groups from the methyl ester of 7 α ,12 α -dihydroxy-3-oxo-5 β -cholanoic acid and 3-oxo-12 α -hydroxy-5 β -cholanoic acid with various solvents using Guttman, Kirkwood-Bayer-Magat model and the Linear relationship of solvation energy.

RESULTS: During the test, the samples were placed in sodium chloride cuvette thickness of 0.057 and 0.116 mm. By recording the FT-IR spectra on the apparatus Thermo Scientific NEXUS 670 and using a detector with deuterated triglicinsulfat in range of 2000 to 1600 cm⁻¹, resolution from 2 cm⁻¹ and 50 scans mode, spectrums were obtained for the concentrations of Me-ester of 7 α ,12 α -dihydroxy-3-oxo-5 β -cholanoic acid and 3-oxo-12 α -hydroxy-5 β -cholanoic acid acid in range from 0.003 to 0.2 moldm⁻³

CONCLUSIONS: The carbonyl from the ester group is more sensitive to interactions with solvents compared with the carbonyl from the keto group, due to less steric protection of carbonyl from the ester functional group. This is a result of higher polarity, caused by inductive effect.

PHENOLIC CONSTITUENTS AND *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF DIFFERENT *RUMEX* SPECIES

Emilija Svirčev¹, Dejan Orčić¹, Kristina Bekvalac¹, Marina Fransišković¹, Ivana Beara¹, Nataša Simin¹, Neda Mimica-Dukić¹

¹ *University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, Novi Sad, Serbia*

*Corresponding author: emilija.svircev@dh.uns.ac.rs

KEYWORDS: *Rumex*; flavonoids; antioxidant; antiinflammatory;

INTRODUCTION: Polygonaceae species are known to be very popular in domain of traditional medicine, cosmetics and food. Most popular species of this subfamily being *Rumex japonicus*, *Polygonum multiflorum*, *Rheum rhabarbarum* do not grow wildly in our country, still there are more than six genera with 43 different species which can be found in Serbia. However, there is no scientific report on chemical composition and biological activities of some species from the subfamily Polygonoideae growing in Serbia.

OBJECTIVES: Considering the fact that the members of family Polygonoideae are significant sources of plant phenols, particularly flavonoids and anthraquinones which exert a variety of biological and pharmacological activities, a hypothesis was carried out that these plant species could be of interest as potential therapeutic agents. On the other hand, based on molecular data, a new tribal classification in Polygonoideae subfamily was suggested, thus chemical analysis for chemosystematic purposes of these species are necessary. Bearing all mentioned in mind, the aim of this study was in-depth phenolic profiling and estimation of antioxidant and anti-inflammatory potential of seven different species belonging to *Rumex* genera insufficiently uninvestigated by now.

METHOD / DESIGN: Phytochemical characterization of prepared herbal and root ethanol extracts from seven different species belonging to *Rumex* genera included spectrophotometric determination of total phenolic, total flavonoid and total anthraquinone contents, quantification of 51 secondary metabolites by LC/MS/MS analysis and *chromatographic fingerprinting* by LC/DAD/MS technique. The antioxidant activity of prepared extracts was evaluated by measuring ferric reducing ability (FRAP) of the extracts and their radical scavenging capacity towards DPPH, OH, NO and O₂⁻ radicals, and inhibition of lipid peroxidation. Anti-inflammatory activity was evaluated by LC/MS/MS monitoring of selected metabolites (12-(S)-HHT, 12(S)-HETE, PGE₂, PGF_{2α}, and TXB₂) formed in cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism. Human platelets were used as a source of enzymes, while inflammation was induced by calcimycin.

RESULTS: Based on obtained results, there is a significant difference in total phenolics, and total flavonoids contents between examined species. Highest content of

total phenolics was found in the herb extracts of *Rumex patientia* and *Rumex crispus* species, while herb extracts of *Rumex acetosella* species were richest in total flavonoid content. The results of quantitative LC-MS-MS analysis of the 51 standard compounds (phenolic acids and derivatives, phenylpropanoids, flavonoids with different degrees of oxidation and anthraquinones) showed that even though the amount of quantified metabolites ranged only from 0.6% to 5.3% of herb extract dry weight and from 0.3% to 6% of rhizome extract dry weight, certain compounds are potential taxonomic markers at the level of subgenera and even at the level of subspecies. Results of qualitative LC-DAD-MS analysis showed that species belonging to subgenus *Rumex* are characterized by the presence of significant amounts of quercetin-3-*O*-glucuronide, musizinin and flavan-3-ol. Subgenera *Acetosa* and *Acetosella* are characterized by the presence of luteolin-*C*-glycosides and apigenin-*C*-glycosides (especially orientin). Species of these two subgenera differ in the fact that *R. acetosa* has significantly greater amount of acetylated *C*-glycosides.

Results obtained by *in vitro* antioxidant assays showed that herb extracts of *R. patientia* possess the best overall antioxidant activity. Rhizome extracts of *R. acetosa* and *R. acetosella* acted as the most selective inhibitors of the COX-1 enzyme. Majority of extracts acted as good inhibitors of 12-LOX pathway. Also, herb extracts, except for *R. balcanicus*, showed stronger activity than the corresponding rhizome extracts.

CONCLUSIONS: In general, the obtained results indicate a high potential for the application of the studied species as medicinal agents and sources of natural, biologically active compounds. It can be concluded that analyzed *Rumex* species can be separated into different subgenera based on obvious differences in chemical composition, and also due to patterned biological activity showed in *in vitro* assays.

ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED BY SUPERCRITICAL EXTRACTION OF YARROW (*Achillea millefolium*) AND ROSE HIP (*Rosa canina*) WASTE PRODUCED IN FILTER TEA FACTORY

Dragana Čučak¹, Senka Vidović², Aleksandra Cvejin², Dragan Radnović³

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia,

² University of Novi Sad, Faculty of Technology, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia,

³ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg D. Obradovića 2, 21000 Novi Sad, Serbia

*Corresponding author: dragana.cucak@dh.uns.ac.rs

KEYWORDS: tea mixtures; antibacterial; antifungal; supercritical extraction; industrial byproducts

INTRODUCTION: Due to the inappropriate and excessive use of antibiotic drugs, increase of antibiotic resistance became one of the most urgent medical issues, calling for development of new alternative antimicrobial agents. Studies have repeatedly shown effectiveness of plant extracts for antimicrobial use. However, profitability of such agents can be fairly limited due to the cultivation and extraction costs. Use of plant industrial waste materials and more effective extraction methods could alleviate this economical issue and increase applicability of plant-based antimicrobial agents.

OBJECTIVES: The aim of this study was to test antimicrobial activity of extracts obtained by supercritical extraction of yarrow (*Achillea millefolium*) and rose hip (*Rosa canina*) “herbal dust” using different bacterial and fungal strains.

METHOD / DESIGN: We used the yarrow and rose hip herbal dust which represents the waste material of filter tea factory. It is material of particle size lower than the pores of filter tea bag, and as such it cannot be used further in the filter tea production. By application of supercritical carbon dioxide, at different temperatures and pressures, various extracts from yarrow and rose hip herbal dust, or their mixtures, were obtained. By standard microdilution broth method MIC and MBC values of extracts against four bacterial and two fungal reference strains were determined.

RESULTS: The results have shown that Gram-positive bacteria were more sensitive in comparison to Gram-negative bacteria (tab. 1) Antibacterial activity increased with the rising percentage of yarrow in the extracts. Highest activity was noted against common food poisoning bacteria *Bacillus cereus*, but also notably low MICs were determined for *Staphylococcus aureus* and *Pseudomonas aeruginosa* - bacteria infamous for their multidrug resistance and nosocomial infections causation. Extracts had no antifungal effect.

Tab. 1 MIC and MBC (MFC) of eight supercritical extracts of tea mixtures of yarrow and rose hip for 6 reference bacterial and fungal strains

Strain		Extract number and percent of yarrow (<i>Achillea millefolium</i>)							
		1 0%	2 20%	3 20%	4 40%	5 60%	6 80%	7 80%	8 100%
<i>Escherichia coli</i> ATCC 25922	MIC	>200	200	>220	52	25,0	25,0	50	25
	MBC	>200	200	>220	>208	50,0	25,0	50	25
<i>Pseudomonas aeruginosa</i> ATCC 9027	MIC	>200	100	≥110	52	50,0	25,0	50	50
	MBC	>200	>200	>220	>208	≥100	>200	50	50
<i>Bacillus cereus</i> ATCC 11778	MIC	100	25	13,75	6,50	6,25	6,25	6,25	6,25
	MBC	100	25	27,50	6,50	6,25	6,25	6,25	6,25
<i>Staphylococcus aureus</i> ATCC 25923	MIC	>200	50	55	26	≥200	6,25	12,50	12,50
	MBC	>200	50	≥220	26	>200	25,00	50	50
<i>Aspergillus niger</i> ATCC 16404	MIC	200	>200	≥220	≥208	≥200	≥200	≥200	≥200
	MBC	>200	>200	>220	>208	>200	>200	>200	>200
<i>Candida albicans</i>	MIC	200	100	≥110	≥104	>200	>200	>200	>200
	MBC	>200	>200	>220	>208	>200	>200	>200	>200

CONCLUSIONS: Remains and extracts of industrial processing of herbs from the filter tea factory have a potential usefulness as antimicrobial agents primarily against certain Gram-positive bacteria. Our findings highlight the need for further research and optimization of extraction procedure to maximize antimicrobial activity and cost effectiveness, as well as for testing of other possible plant mixtures.

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF *CALAMINTHA* MILL. SPECIES (LAMIACEAE)

Marina Milenković¹, Jelena Stošović¹, Violeta Slavkowska²

¹Department of Microbiology and Immunology, University of Belgrade-Faculty of Pharmacy,

²Department of Botany, University of Belgrade-Faculty of Pharmacy

*Corresponding author: violetas@pharmacy.bg.ac.rs

KEYWORDS: essential oils; antibiotics; *Calamintha sylvatica*; *C. vardarensis*; *C. nepeta*; *C. glandulosa*

INTRODUCTION: The necessity for finding new antimicrobial agents and approaches in curing infections caused by resistant bacterial strains increases every day. One of the approaches to this problem is to use essential oils as antibiotic adjuvants, with the aim of achieving synergistic interactions.

OBJECTIVES: In this work we examined the antimicrobial activity of the essential oils (EOs) of *Calamintha sylvatica* Bromf. *C. vardarensis* Šilić, *C. nepeta* (L) Savi and *C.glandulosa* (Req.) Benth., as well as their antibacterial activity in combination with antibiotics.

METHOD / DESIGN: Essential oils were obtained by hydrodistillation in the apparatus by Clevenger according to Ph. Eur. 7. Quantitative and qualitative analysis of the essential oils was performed using gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). Broth microdilution method was used to test the antimicrobial activity of the tested EOs against six standard bacterial strains: *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella abony* (ATCC 6017), and against one strain of yeast: *Candida albicans* (ATCC 10231). Antibacterial activity of the EOs in combination with the antibiotics gentamicin and ciprofloxacin was examined by checkerboard method against: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella abony*.

RESULTS: The dominant components of the essential oil of *C. sylvatica* were: *cis*-piperitone epoxide (63.3%) and menthone (10.8%), of *C. vardarensis* oil: pulegone (51.6%), menthone (19.9%) and *cis*-piperitone epoxide (7.1%), of *C. nepeta* oil: pulegone (58%) and piperitenone (27.4%), and of *C. glandulosa* oil: pulegone (35.1%), piperitenone (23.4%), menthone (15.7%) and piperitone (11.5%).

The antimicrobial activity of the EOs is expressed through minimal inhibitory concentration (MIC) values, which are: for essential oil of *C. sylvatica* >182 µg/ml, *C. nepeta* >191 µg/ml and for the oil of *C. glandulosa* >186 µg/ml for all tested strains. MIC values for the essential oil of *C. vardarensis* are >170 µg/ml for all tested strains, except for *S. aureus* whose MIC value is 21.25 µg/ml. The results of the checkerboard assay, interpreted by fractional inhibitory concentration (FIC) index, show different effects

depending on the tested strain. Combination of EOs of *C. sylvatica*, *C. vardarensis* and *C. nepeta* with gentamicin/ciprofloxacin has shown synergistic effect against *S. abony*. Likewise synergistic effect is achieved by the combined action of the essential oils of *C. nepeta* and *C. glandulosa* with gentamicin against *S. aureus*. Essential oils of *C. sylvatica* and *C. vardarensis* in combination with ciprofloxacin were shown to have synergistic effect against *E. coli*, while the oil of *C. nepeta* has shown the same effect against *P. aeruginosa*.

CONCLUSIONS: Based on the determined MIC values, we can conclude that the tested EOs did not exhibit significant antimicrobial activity. In contrast, it has been found that EOs in combination with antibiotics show different effects (from synergistic to antagonistic) depending on the tested strain. Essential oil of *C. nepeta* has shown higher efficiency in combination with antibiotics.

SYNTHESIS AND 17 β -HSD1 INHIBITION OF NOVEL 2- OR 4-SUBSTITUTED 13 α -ESTRONE DERIVATIVES

Rebeka Jójárt¹, Ildikó Bacsa¹, János Wölfling¹, Gyula Schneider¹,
Bianka Edina Herman², Mihály Szécsi², Erzsébet Mernyák¹

¹ Department of Organic Chemistry, University of Szeged, Dóm tér 8, H6720 Szeged, Hungary

² 1st Department of Medicine, University of Szeged, Korányi fasor 8-10, H6720 Szeged, Hungary

*Corresponding author: rrebek@gmail.com

KEYWORDS: 13 α -estrone; Sonogashira coupling; 17 β -HSD1 inhibition.

INTRODUCTION: We published recently the syntheses and *in vitro* biological evaluations of several 13 α -estrone derivatives. Electrophilic brominations or iodinations were carried out, furnishing 2-, 4- or 2,4-*bis*-halogenated compounds. All the halogenated 3-hydroxy and the 4-regioisomers of 3-methyl ethers displayed substantial inhibitory action against 17 β -hydroxysteroid dehydrogenase type 1 enzyme (17 β -HSD1), which is responsible for the stereospecific reduction of prehormone estrone into 17 β -estradiol. These halogenated derivatives, beside their pharmacological importance, may serve as appropriate starting compounds for Pd-catalyzed C-C coupling reactions.

OBJECTIVES: Here we aimed to develop facile and effective Sonogashira coupling methods for the preparation of biologically active 2- or 4-phenethynyl derivatives in the 13 α -estrone series. 2-Iodo- or 4-iodo-13 α -estrone and their 3-methyl ethers were chosen as starting compounds.

METHOD / DESIGN: The optimizations of the coupling reactions were carried out using phenylacetylene as a model reagent and Pd(PPh₃)₄ or Pd(PPh₃)₂Cl₂ as a catalyst. The structures of the new compounds were confirmed by ¹H and ¹³C NMR measurements. The potential inhibitory action of the novel 13 α -estrones on human 17 β -HSD1 activity was investigated via *in vitro* radiosubstrate incubation.

RESULTS: The Sonogashira reactions (from both regioisomers) were carried out with several *p*-substituted phenylacetylenes. It was found that the optimal reaction conditions differ depending on the position of the iodine on the sterane skeleton. All the couplings resulted in the desired product with high yields. Some compounds proved to be effective 17 β -HSD1 inhibitors, with IC₅₀ values in the low micromolar range.

CONCLUSIONS: Further derivatization of the promising 13 α -estrone scaffold may lead to drug candidates that possess substantial endocrine disruptor behavior.

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CHEMICAL DIVERSITY OF PRIMARY VOLATILE COMPOUNDS IN GARLICS (*ALLIUM* L., SECT. *ALLIUM*, ALLIACEAE)

Biljana Božin¹, Nebojša Kladar¹, Neda Gavarić¹, Dejan Orčić²,
Neda Mimica-Dukić², Goran Anačkov²

¹University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia

²University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, Novi Sad, Serbia

*Corresponding author: biljana.bozin@mf.uns.ac.rs, bbozin2003@gmail.com

KEYWORDS: *Allium* L.; chemical diversity; volatile sulfur compounds; medicinal plant

INTRODUCTION: Garlic (*Allium sativum* L., Alliaceae) is used as a flavoring in salads and different meals. As a vegetable it is used as an immature plants. However, it is also widely accepted as a herbal medicine for the treatment of high LDL cholesterol, as an antiatherosclerotic, hypoglycaemic, anticoagulant, antihypertensive and natural antibiotic. Generally, it is considered that the health benefits are mostly attributed to the presence of γ -glutamylcysteine in the fresh plants, along with the primary volatile sulfur-containing components responsible for a characteristic flavour formed during processing of bulbs or leaves. But, many other representatives of the section are not investigated in detail, although they are used as an alternative for common garlic.

OBJECTIVES: The aim of this study was to evaluate the chemical diversity of primary volatile sulfur compounds among 10 different “garlic” taxa (*Allium sativum* var. *vulgare*, *A. scorodoprasum*, *A. rotundum* subsp. *waldsteinii*, *A. sphaerocephalon*, *A. vineale* var. *compactum*, *A. vineale* var. *vineale*, *A. vineale* var. *capsuliferum*, *A. guttatum* subsp. *dalmaticum*, *A. guttatum* subsp. *sardoum* and *A. atrovioleaceum*) collected at 15 localities in Republic of Serbia. Voucher specimens of the collected plant material were identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS), Faculty of Natural Sciences, University of Novi Sad.

METHOD / DESIGN: Qualitative and quantitative analysis of the volatile sulfur compounds formed after the disruption of cellular structures in the different organs of the studied taxa (bulbs, leaves and/or inflorescences) were carried out using a Hewlett-Packard 5973-6890 Headspace gas chromatography-mass spectrometry (GC/MS) system. For identification, three libraries of mass spectra were used: Wiley Registry of Mass Spectral Data 7th Edition, NIST/EPA/NIH Mass Spectral Library Ver. 05, and an Agilent Technologies Flavor2 Library. All the data obtained during the study were processed using software package Microsoft Excel for Windows, v2007 and Statistica for Windows, Ver. 12.

RESULTS: Headspace-GC/MS analysis of investigated taxa pointed on significant differences in the chemical composition of volatile compounds, especially related to the different organs and the plant phenophase. The persistence of notable differences is

confirmed also in investigated taxa among selected representatives on intraspecific level (*A. guttatum* and *A. vineale*), as well as several chemotypes in the varieties subjected to the study.

CONCLUSIONS: Although only garlic (*A. sativum*) is widely used in human nutrition, but also in traditional medicine for centuries among the investigated taxa, there are several chemically very similar representatives in the Sect. *Allium*. They could be of potential importance for pharmaceutical and medicinal use, especially after the notification of several side effects and herb-drug interactions confirmed for common garlic.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF BILE ACID ETHYLIDENE AMIDES AND OXAZOLINES

Srdjan Bjedov¹, Dimitar Jakimov², Marija Sakač¹

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, Novi Sad, Serbia,

² Oncology Institute of Vojvodina, Institutski put 4, Sremska Kamenica, Serbia

*Corresponding author: srdjan.bjedov@dh.un.ac.rs

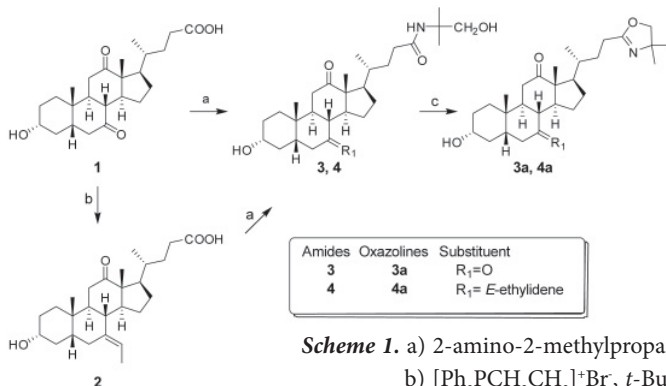
KEYWORDS: bile acid; oxazolines; amides; antitumor agents

INTRODUCTION: In addition to the known physiological function of bile acids in the transport of lipids, fat-soluble vitamins and the elimination of cholesterol from the body, recent studies¹ reveal hormonal role of bile acids. Henodeoxycholic acid is the natural ligand for the nuclear receptor FXR and for membranotropic, TRG5 receptor that participate in the lipid, carbohydrate and energy homeostasis. Synthetic, bile acid alkyl derivatives are agonists of FXR TRG5 receptors,² and bile acid amides and oxazoline derivatives have promising biological potential.

OBJECTIVES: Our objectives were synthesis of bile acid derivatives with ethylidene chain on steroid skeleton, and functionalization of carboxylic group to amide and oxazoline group. Important part is investigation of biological activity these bile acid ethylidene, amide or oxazoline derivatives.

METHOD / DESIGN: Bile acid ethylidene derivatives are synthesized utilizing appropriate oxo derivatives in Wittig reaction. Bile acids were transformed into amides using carboxylic group activator EEDQ, and oxazolines were obtained by cyclization of amides using thionyl chloride.

RESULTS: We have successfully synthesized bile acid ethylidene amides **4** and oxazolines **4a** (Scheme 1.). Cytotoxic activities of compounds **2**, **3**, **4**, **3a** and **4a** were investigated *in vitro* against several cancer cell lines and one normal cell line (Table 1.).



Scheme 1. a) 2-amino-2-methylpropanol, EEDQ, EtOAc;

b) [Ph₃PCH₂CH₃]⁺Br⁻, *t*-BuOK, THF; c) SOCl₂, THF.

	MCF-7	MDA-MB-231	PC3	HeLa	MRC-5
2	72.15	1.07	>100	>100	>100
3	16.46	>100	8.58	>100	>100
4	63.81	1.79	11.90	4.07	30.08
3a	78.92	>100	10.62	12.89	>100
4a	85.65	5.85	20.48	17.15	32.80

Table 1. IC₅₀ (μM), 72 h

CONCLUSIONS: Some of newly synthesized bile acid derivatives showed good to very potent cytotoxic activity. Especially potent were 7-ethylidene derivatives **2**, **4** and **4a** against MDA-MB-231 cell line, and **4a** against HeLa cell line with IC₅₀ values under 10 μM. These findings have shown that bile acid ethylidene derivatives may have important pharmaceutical potential.

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VITIS VINIFERA LEAF EXTRACTS – PHENOLIC PROFILE, ANTIOXIDANT AND NEUROPROTECTIVE PROPERTIES

Diandra Pintac¹, Tatjana Majkić¹, Sanja Berić¹, Jelena Nađpal¹, Neda Mimica-Dukić¹, Ivana Beara¹, Marija Lesjak¹

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

*Corresponding author: diandra.pintac@dh.uns.ac.rs

KEYWORDS: *Vitis vinifera* leaves; acetylcholinesterase; antioxidant activity; phenolic profile;

INTRODUCTION: Although *Vitis vinifera* is most commonly cultivated for obtaining grapes and its products, leaves of grapevine are also widely used in the Mediterranean diet, Ayurveda and in the folk medicine of many countries. Their beneficial effect on human health has been recognized in treating diarrhea, vomiting, hemorrhage, hypertension, circulatory system diseases and inflammatory disorders. However there is still not enough data on their chemical profile and biopotential.

OBJECTIVES: Since phenolics are mainly responsible for biopotential of plants, the aim of this study was to determine a detailed phenolic profile, as well as antioxidant and neuroprotective activity of three leaf extracts of white grape varieties – Chardonnay, Sila and Župljanka, grown in the vineyards of Fruška Gora, Serbia.

METHOD / DESIGN: Leaf extracts were prepared by extraction of air-dried leaves with 80% methanol, washing with petrol ether (fraction 40–60°C) to remove nonpolar compounds, drying of the crude extracts under vacuum and dissolving in water. Quantitative analysis of 45 phenolic compounds was performed by the LC-MS/MS technique. Antioxidant potential was evaluated with standard spectrophotometric tests by measuring free radical scavenging ability of the samples towards diphenylpicrylhydrazyl (DPPH[•]), superoxide anion (O₂^{•-}) and hydroxyl (HO[•]) radicals. Acetylcholinesterase (AChE) inhibition assay was conducted in order to elucidate a potential neuroprotective effect of the leaf extracts.

RESULTS: From 45 examined phenolic compounds, 21 were found to be present in the leaves above the quantification limit, among which quercetin-3-O-glucoside was the most abundant flavonoid in all samples (Chardonnay: 4.77 mg/L; Župljanka: 5.62 mg/L; Sila: 3.41 mg/L). As for the phenolic acids, Župljanka and Sila leaf extracts were rich in chlorogenic acid (0.66 and 0.44 mg/L, respectively), while in Chardonnay vanillic acid was dominant (0.10 mg/L). Chardonnay leaf extract expressed a strong scavenging of DPPH[•] and HO[•], while the highest inhibition of AChE and scavenging of O₂^{•-} was achieved by the extract of Župljanka. All samples showed a moderate activity compared to a well known synthetic antioxidant – propyl gallate, and a standard inhibitor of AChE – galantamine, with the exception of O₂^{•-} scavenging assay where the three leaf extracts were more potent than PG.

CONCLUSIONS: Presented results indicate a good therapeutic potential of leaves of white grape varieties grown in Fruška Gora which exhibited strong antioxidant and neuroprotective properties. This research introduces novel data on the phenolic profile of three Serbian grapevine leaf varieties. Thanks to their high nutritional value, this study supports the use of fresh *Vitis vinifera* leaves in diet. Also, the utilization of grape vine leaves that remain as a by-product during winemaking could greatly benefit the food and pharmaceutical industry, as they are a rich and affordable source of phenolic compounds.

EFFECTS OF SHORT-TERM CHRONIC LARD- OR PALM OIL CONSUMPTION ON VASCULAR FUNCTIONS IN MIDDLE-AGED MALE RATS

Chaweewan Jansakul^{1,4}, Vatchara Chongsa^{1,4}, Chula Wiriyabubpha¹, Jomkarn Naphatthalung^{1,4}, Sakda Pradab^{1,4}, Kanyanatt Kanokwiroon^{2,5}, Nisaudah Radenahmad³

¹ Faculty of Traditional Thai Medicine,

² Department of Biomedical Sciences, Faculty of Medicine,

³ Department of Anatomy, Faculty of Science,

⁴ Natural Product Research Centre of Excellence and

⁵ The Excellent Research Laboratory of Cancer Molecular Biology, Prince of Songkla University, Hat-Yai, Thailand

*Corresponding author: Chaweewan.j@psu.ac.th

KEYWORDS: Lard oil; palm oil; NO; H₂S; middle-aged rat; thoracic aorta

INTRODUCTION: Lard oil, according to Thai wisdom, has been used for cooking in Thai Cuisine since ancient times. In the last 3 decades, lard oil has been replaced by palm oil mainly because of the notion that plant oil consumption would be better for cardiovascular health than animal fat. However, scientific information to support this notion is still controversial.

OBJECTIVES: To investigate whether after 6 weeks of feeding middle-aged male rats with either lard- or palm oil would affect animal blood pressure and vascular functions.

METHOD / DESIGN: Lard oil, palm oil or distilled water (control) at a dosage of 1 and 3 ml/kg were orally gavaged once a day for 6 weeks to rats. At the end of treatment, each animal was measured basal blood pressure and heart rate with a Polygraph. Fasting blood sugar and lipids were measured by enzymatic methods. The vascular functions of isolated thoracic aorta were studied using pharmacological techniques and measurements of vascular eNOS protein expression by Western blotting.

RESULTS: At the moderated amount (1 ml/kg) of lard- or palm oils treatment to middle-aged rat there were no differences observed on basal blood pressure and heart rate, either to any other parameters studies. However, when the dosage was increased to 3 ml/kg, lard oil caused an increase in basal blood pressure, body fat and liver cell lipid accumulation, and plasma ALP and SGPT levels, whereas palm oil only caused an increase in the fasting serum triglyceride level compared to the control group. At the dosage of 3 ml/kg, both of lard- and palm oil treatment had no effects on thoracic aortic ring responsiveness to phenylephrine, acetylcholine or glyceryl trinitrate. Palm oil, but not the lard oil treatment caused a decrease in eNOS protein expression compared to that of the control group. Both the lard- and palm oil treatment caused an increase in the basal vascular H₂S production as DL-propargylglycine, an H₂S inhibi-

tor, caused an increase in the baseline tension of the aortic ring, and as a result there was an increase in the contractile response to phenylephrine at low concentrations of phenylephrine.

CONCLUSIONS: Consumption of lard or palm oil in moderation (1 ml/kg) for 6 weeks caused no significant deleterious effects associated with the development of cardiovascular disease. However when the middle aged rats were fed with 3 ml/kg of the lard oil, there was an increased risk for the development of cardiovascular disease and/or on the metabolic syndrome.

IN VITRO AND IN VIVO ANTIOXIDANT POTENTIAL OF ESSENTIAL OIL OF ANISEED (*PIMPINELLA ANISUM* L. APIACEAE)

Isidora Samojlik¹, Biljana Božin², Neda Gavarić², Vesna Mijatović¹, Stojan Petković³

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology,

² University of Novi Sad, Faculty of Medicine, Department of Pharmacy,

³ University of Novi Sad, Faculty of Medicine, Department of Forensic Medicine, Novi Sad, Serbia

*Corresponding author: isidora.samojlik@mf.uns.ac.rs

KEYWORDS: *Pimpinella anisum*; essential oil; antioxidant properties; DPPH; lipid peroxidation

INTRODUCTION: Anise (*Pimpinella anisum* L.; Apiaceae) and its essential oil (EO) have been widely used in traditional medicine, pharmacy and food industry. Although *in vitro* antioxidant properties of various anise extracts and EO are recorded, there is little evidence of such *in vivo* effect.

OBJECTIVES: This survey aimed to examine the aniseed EO *in vitro* antioxidant effect together with its *in vivo* activity evaluated through hepatoprotective effect in damage caused by carbon tetrachloride (CCl₄).

METHOD / DESIGN: The *in vitro* antioxidant capacity was evaluated as a free radical scavenging capacity (RSC). It was measured as scavenging activity of the EO on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]) and through effects on lipid peroxidation (LP) in two systems of induction. Several liver biochemical parameters (reduced glutathione – GSH, peroxidase –Px, catalase – CAT, glutathione peroxidase – GSH-Px, lipid peroxidation LPx) were determined in animals (mice) pre-treated with the EO during 5 consecutive days and later intoxicated with CCl₄ to assess *in vivo* antioxidant – hepatoprotective effect.

RESULTS: The composition of EO determined by GC-MS confirmed *trans*-anethole (88.49%) as main component followed by γ -himachalene (3.13%), *cis*-isoeugenol (1.99%) and linalool (1.79%). Anise EO was able to reduce the stable DPPH[•] in a dose-dependent manner reaching 50% neutralization with IC₅₀ value of 11.25 mg/ml and to inhibit LP in both systems of induction. *In vivo* investigation revealed dominant oxidative processes in liver after the EO pre-treatment (decrease in GSH and the increase of LPx and Px activity) and the leak of antioxidative capacity after CCl₄ intoxication (depletion of GSH, decrease in CAT and GSH-Px activity and preserved increase of LPx).

CONCLUSIONS: Although *in vitro* antioxidant potential of aniseed EO was demonstrated, *in vivo* investigation indicated that caution should be included when the anise-flavoured drinks or anise-containing medicines are consumed together with drugs that need or decrease GSH in its metabolism. Further clinical relevance still remains to be studied.

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ANTIPROLIFERATIVE EFFECT OF 19-NORTESTOSTERONE ANALOGUES ON GYNECOLOGICAL CANCER CELL LINES

András Gyovai¹, Renáta Minorics¹, Noémi Bózsity¹, Anita Kiss², Imre Ocsovszki³, Gyula Schneider², István Zupkó¹

¹ Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

² Department of Organic Chemistry, University of Szeged, Szeged, Hungary

³ Department of Biochemistry, University of Szeged, Szeged, Hungary

*Corresponding author: András Gyovai: gyovai.andras@pharm.u-szeged.hu

Keywords: cancer cell, apoptosis, androstane

Introduction: Anticancer effects of several compounds with androstane skeleton have been reported recently. The aim of this work was to investigate a set of novel 19-nortestosterone derivatives and their antitumor activity against human adherent cell lines. The antiproliferative effect of the compounds was determined. In the case of most potent analogue, the tumor selectivity and the mechanism of action were investigated.

Methods: The antiproliferative effect of the 19-nortestosterone derivatives was determined by means of MTT assay on ovary-, cervical- and breast carcinoma cell lines. The tumor selectivity of the effective analogues was examined by repeated assay on non-cancerous fibroblast cells. The most effective compound was selected for further experiments. The cytotoxic effect of the most potent analogue was measured by LDH assay. The induction of apoptosis was detected by fluorescent microscopy and measurement of caspase activity. The alterations of cell cycle were investigated by means of flow cytometry. The influence on microtubule system by the selected compound was determined using tubulin polymerization assay.

Results: Three compounds (17 α -chloro- (**1**), 17 α -bromo- (**2**), 17 α -iodo-19-nortestosterone (**3**)) showed a potent antiproliferative effect on HeLa cells (IC₅₀: 1.2–1.7 μ M) with promising tumor selectivity (IC₅₀ > 30 μ M, fibroblast). The most potent analogue (**1**: IC₅₀: 1.2 μ M) can cause moderate membrane damage and cell cycle arrest in the case of lower concentration due to accumulation of the cells in S or G2/M phase. The increase of sub-diploid population (SubG1) suggests the compound mediated induction of apoptosis.

The induction of programmed cell death by the tested analogue is evinced by morphological examinations. The induction of intrinsic pathway of programmed cell death has been proved by increasing activities of caspase-3 and -9 with unchanged caspase-8 activity.

The most potent compound has a directly influence on tubulin polymerization due to increase of the velocity of polymerization in vitro.

CONCLUSIONS: According to our results the C17 α substitution of the 19-nortestosterons with halogens is responsible for the antiproliferative effect. The most potent compound is capable of inducing apoptosis and has a temperate cytotoxic effect. This agent is able to cause a disturbance of cytoskeleton due to its influence on tubulin polymerization which may be responsible for both of cell cycle arrest and the induction of apoptosis.

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ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF BLACKTHORN GENOTYPES

Dubravka Štajner¹, Boris Popović¹, Ana Teresa Serra², Ružica Ždero Pavlović¹, **Bojana Blagojević¹**, Catarina M.M. Duarte²

¹ Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

² Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Av. da Republica, EAN, 2780-157 Oeiras, Portugal

*Corresponding author: bojana.blagojevic@polj.uns.ac.rs

KEYWORDS: blackthorn; phenolics; antioxidant activity; antiproliferative activity

INTRODUCTION: Blackthorn (*Prunus spinosa* L.), which belongs to the rose family (*Rosaceae*), is a perennial plant growing as a shrub on slopes of wild uncultivated areas. It is known to have many health-promoting activities, especially anticancer, antiradical and antioxidant effects. Most of these actions are believed to be due to its polyphenolic components.

OBJECTIVES: The objective of this study was to evaluate antiproliferative activity, antioxidant capacity and phenolic content in fruits of six different genotypes of *P. spinosa*, grown in south Bačka region.

METHOD / DESIGN: Fruits of six different blackthorn genotypes were assessed: B1, B2, BL1, BL2, TR-MB/1, TR-MB/2. Fresh fruits were freeze dried and grinded prior to extraction. Total phenolic, flavonoid and anthocyanin contents of 50% acid methanol (1% HCOOH) extracts were quantified by spectrophotometrical methods (Folin-Ciocalteu, aluminium chloride and pH differential method, respectively). In order to detect antioxidant activity, a method based on the reduction of DPPH radical and ferric reducing antioxidant power (FRAP) method were carried out. Antiproliferative cell assays were performed using human colon cancer cells HT29.

RESULTS: Genotypes BL1 and BL2 showed the greatest values of all determined parameters. Total phenolics varied from 42.89 to 17.03 mgGAE/gDW, with genotype BL2 with the highest, and TR-MB/2 with the lowest content. Genotype BL1 showed the highest content of total flavonoids (5.84 mgQE/gDW) and anthocyanins (5.82 mgC3G/gDW), more than twice higher than in genotypes TR-MB/1 and TR-MB/2. Extracts of all genotypes showed remarkable antioxidant and antiproliferative activities, highlighting genotypes BL1 and BL2 with the highest, B1 and B2 genotype with moderate and TR-MB/1 and TR-MB/2 with the lowest activities.

CONCLUSIONS: Very strong antioxidant and antiproliferative activities of blackthorn are directly related to content of total phenolics, flavonoids and anthocyanins. All examined blackthorn genotypes demonstrated to be rich sources of functional ingredients and promising raw material for the production of bioactive extracts.

COMPARISON OF ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF FOUR *PRUNUS* SPECIES TRADITIONALLY GROWN IN SERBIA

Dubravka Štajner¹, Boris Popović¹, Ana Teresa Serra², Ružica Ždero Pavlović¹, **Bojana Blagojević¹**, Catarina M.M. Duarte²

¹ Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

² Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da Republica, EAN, 2780-157 Oeiras, Portugal

*Corresponding author bojana.blagojevic@polj.uns.ac.rs

KEYWORDS: *Prunus*; antioxidant activity; antiproliferative activity; phenolics

INTRODUCTION: Regular consumption of fruits has been associated with reduced risk of chronic diseases. These benefits are often attributed to their high content of phytochemical, such as phenolics, with health benefiting effects. *Prunus* is a genus of trees and shrubs, which includes plums, cherries, peaches, apricots and other stone fruits, which are widely consumed in human diet and present a great source of valuable phytochemicals.

OBJECTIVES: Evaluation and comparison of antioxidant capacity, antiproliferative activity and phenolic content of fruits of different *Prunus* species grown in south Bačka region, Vojvodina, Serbia.

METHOD / DESIGN: Extracts of lyophilized fruits of four different *Prunus* species were assessed: sweet cherry (*P. avium*; wild type), plum (*P. domestica*; cultivar Čačanska rodna), apricot (*P. armeniaca*; cultivar DM) and blackthorn (*P. spinosa*, genotype BL2).

Total phenolic, flavonoid and anthocyanin contents were quantified by Folin-Ciocalteu, aluminium chloride and pH differential colorimetric methods, respectively. Total antioxidant activity was determined using scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical assay and measuring the ferric reducing activity power. Antiproliferative effect was determined against human colon HT29 cancer cells.

RESULTS: Extracts of all fruits showed good antioxidant and antiproliferative activities, but blackthorn was distinguished as the most potent. It showed two times higher antiproliferative activity than apricot and approximately four times higher than plum and cherry extracts. Concerning total phenolic, flavonoid and anthocyanin contents, apricot had the lowest values. Blackthorn fruits were the richest in phenolic and flavonoid contents, while sweet cherry had much higher content of total anthocyanins than other examined species.

CONCLUSIONS: These preliminary results could help justifying traditional use of these examined fruits as therapeutics. Furthermore, among *Prunus* species examined in this study, blackthorn showed the best potential to be used as a source of functional ingredients.

CHEMOMETRIC APPROACH OF ANTIOXIDANT PROFILE OF SWEET BASIL

Branislava Rakić^{1,2}, Nevena Grujić-Letić¹, Svetlana Goločorbin-Kon¹, Aleksandar Rašković¹ and Zorica Mrkonjić^{2,3}

¹ University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia,

² University of Business Academy in Novi Sad, Faculty of Pharmacy, Novi Sad, Trg Mladenaca 5, 21000 Novi Sad, Serbia,

³ University of Novi Sad, Faculty of Science, Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia

*Corresponding author: rakic.branislava@gmail.com

KEY WORDS: Antioxidant activity; basil; chemometric analysis; phenols; flavonoids

INTRODUCTION: Healthy lifestyle has great impact on well-being due to possibility to prevent nutrition-related diseases which include scavange of free radicals and block free radicals-chain reactions. Basil (*Ocimum basilicum* L.) contains many antioxidant substances which contribute to its intense antiradical activity and could have potential human health benefits.

OBJECTIVES: The aim of the work is to test the antioxidant activity of basil extracts obtained from different polar (water, methanol, different concertation of ethanol) and non-polar (chloroform, dichloromethane, hexane) solvents.

METHOD / DESIGN: The total phenolic and flavonoid contents were analyzed by spectrophotometric methods and expressed as milligram of gallic acid equivalents on gram of dry extract (mg GAE/g SE) and milligram of quercetin equivalents on gram of dry extract (mg QE/gSE), respectively. The antioxidant activity was determined by a spectrophotometric method based on the reduction of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed.

RESULTS: Total phenol content ranged from 5.17 to 65.25 mg GAE/gDE, and the content of total flavonoids from 0.11 to 40.63 mg QE/gDE. All extracts showed good antioxidant activity with an IC₅₀ value in the range from 0.22 to 20.49 µg/ml. The strongest antioxidant activity was showed after maceration with 60% ethanol (v/v) in 72 hours. It could be generally noted that the antioxidant capacity was depended on the solvent polarity.

CONCLUSIONS: Chemometric analyses were found that increased time of extraction, solvent polarity and degree of comminution of the drug increase the quality of the extracts in terms of the content of phenolic components and antioxidant effects.

CHEMICAL COMPOSITION AND FREE RADICAL SCAVENGING ACTIVITY OF NON-POLAR BASIL EXTRACTS

Branislava Rakić^{1,2}, Nevena Grujić-Letić¹, Svetlana Goločorbin-Kon¹, Aleksandar Rašković¹, Zorica Mrkonjić^{2,3} and Srđan Stojanović²

¹ University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

² University of Business Academy in Novi Sad, Faculty of Pharmacy, Novi Sad, Trg Mladenaca 5, 21000 Novi Sad, Serbia

³ University of Novi Sad, Faculty of Science, Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia

*Corresponding author: rakic.branislava@gmail.com

KEY WORDS: Antioxidant activity; basil; phenols; flavonoids

INTRODUCTION: Huge interests in functional food are due to their low cost, effective approach and relatively safe application. Phenolics and flavonoids isolated from plants are one of the groups of functional ingredients which can enhance health, increase psychical and mental well-being and scavenge free radicals. Basil (*Ocimum basilicum*, L) has a strong antioxidant activity because of a great amount of phenolic and flavonoid components.

OBJECTIVES: The aim of the work is to investigate the antioxidant activity and chemical characterization of basil extracts obtained with different non-polar solvents.

METHOD / DESIGN: The extraction was performed with dichloromethane, chloroform and hexane during different periods of time (10 and 30 minutes) and the degree of fragmentation was 0.3 and 2 mm. The total phenolic and flavonoid contents were investigated by spectrophotometric methods and expressed as mg of gallic acid equivalents on g of dry extract (mg GAE/g SE), and mg of quercetin equivalents on g of dry extract (mg QE/g SE), respectively. The antioxidant activity was determined by a spectrophotometric method based on the degree of inhibition of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Chemical characterization was carried out using high performance liquid chromatography (HPLC).

RESULTS: Total phenol content ranged from 6.56 to 28.91 mg GAE/g SE and the content of total flavonoids from 3.73 to 40.62 mg QE/g SE. All extracts showed relatively good antioxidant activity with an IC₅₀ value in the range from 12.12 to 20.49 µg/ml. The best values were obtained after maceration with chloroform during 30 minutes. The extracts contained chlorogenic, *p*-hydroxybenzoic, caffeic, ferulic, vanillic, rosmarinic and cinnamic acid, rutin, quercetin and naringenin.

CONCLUSIONS: The extraction conditions showed a significant effect on the chemical composition of the obtained extracts. It was found that increased time of extraction, solvent polarity and degree of comminution of the drug increase the quality of the extracts in terms of the content of phenolic components and antioxidant effects.

EXTRACTION OF BIOACTIVE COMPOUNDS FROM *PARKIA SPECIOSA* HASSK. AND *COTYLELOBIUM MELANOXYLON* PIERRE. MIXTURES

Wanhayatee Kasaw¹, Punnanee Sumpavapol¹, Bhudsawan Hiranvarachat²

¹ Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hatyai, Songkhla, 90112 Thailand.

² Department of Agro-Industrial Technology, Faculty of Agro-Industry, Prince of Songkla University, Hatyai, Songkhla, 90112 Thailand.

*Corresponding author: punnanee.s@psu.ac.th

KEYWORDS: extraction; phenolic compounds; flavonoid; biological activity; environmental impact

INTRODUCTION: Nowadays, various food industry generate large amount of by-products that are often underutilized, which could have negative environmental impact and increase the price of the raw material as it is not completely utilized in the production. Pods of *Parkia speciosa* Hassk. (stink bean) and leaves of *Cotylelobium melanoxylon* Pierre. (*Kiam*) considered as industrial waste compose of diverse bioactive compounds such as phenolic compounds, flavonoids and tannins, knowing to possess various biological activities such as antioxidant and antimicrobial (Wonghirundecha and Sumpavapol, 2012; Wonghirundecha, 2013).

OBJECTIVES: Mixture of two interesting plants (stink bean pod and *Kiam* leaf) obtained as by-products with different ratios have been extracted. In the obtained extracts, quantitative content of phenolic compounds and flavonoid and biological activities such as antioxidant and antimicrobial were determined and compared.

METHOD/DESIGN: Plant mixture (stink bean pod and *Kiam* leaf) at the ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 (w/w) were extracted with ethanol by conventional technique (Wonghirundecha *et al.*, 2014). Total phenolic content and total flavonoid content were evaluated according to Folin-Ciocalteu procedure and aluminum chloride colorimetric assays, respectively. Antioxidant activity of obtained extracts was determined by measuring free radical scavenging activity (ABTS – test) and reducing power (FRAP- assays), while antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was evaluated by agar-well diffusion assay.

RESULTS: Stink bean pod extract showed the highest contents of total phenolic and total flavonoid. Therefore, total phenolic and flavonoid contents of plant mixture extract increased when increasing stink bean pod content ($p < 0.05$). High correlation between total phenolic and flavonoid contents and biological activities was observed. On that account, stink bean pod extract exhibited the strongest activities. Accordingly, antioxidant and antibacterial activities increased when the stink bean pod content increased. Exceptional was found in antibacterial activity against *Staphylococcus aureus* where *Kiam* leaf extract showed the strongest activity. The results showed that stink

bean pod, which regarded as industrial waste, could be used as alternative source for extraction of bioactive compounds.

CONCLUSIONS: Utilization of agriculture waste provided value-added products, rich in various health benefit compounds. However, traditional technology used for extraction of bioactive compounds are no longer environmentally acceptable and alternative green technologies, such as ultrasonic-assisted extraction and microwave-assisted extraction, should be considered. Thus, further study will be focus on extraction of bioactive compounds from stink bean pod by green technology.

ANTIOXIDANT ACTIVITY AND CYTOTOXIC POTENTIAL OF SELECTED COMMERCIAL SPICES

Blagica Jovanova¹, Svetlana Kulevanova² and **Tatjana Kadifkova Panovska**¹

¹ Faculty of Pharmacy, Institute of Applied Biochemistry, Ss. Cyril and Methodius University, Majka Tereza 47, Skopje, Republic of Macedonia

² Faculty of Pharmacy, Institute of Pharmacognosy, Ss. Cyril and Methodius University, Majka Tereza 47, Skopje, Republic of Macedonia

*Corresponding author: blagicajovanova@hotmail.com

KEYWORDS: DPPH; total phenolic content; brine shrimp lethality assay; LC₅₀; spices.

INTRODUCTION: Many commercial spices are regularly used as part of the nutrition of the population. Their wide usage in diet is already exposing the population to the benefits of the culinary herbs, but also to compounds that beside their medicinal properties could manifest toxic effects.

OBJECTIVES: The aim of this study is to determine the antioxidant activity and the cytotoxic potential of the selected spices: *Origanum majorana*, *Carthamus tinctorius* and *Sinapis alba*.

METHOD / DESIGN: The extracts were prepared by ultrasonification of the dry plant material with 96% ethanol. The antioxidant activity was evaluated with the DPPH (2,2'-diphenyl-1-picrylhydrazyl) assay, and expressed as LC₅₀ values. Additionally, the total phenolic content of the extracts was determined by Folin-Ciocalteu method, while the total flavonoid content was determined by the AlCl₃ method.

The cytotoxic potential of the ethanol extracts was evaluated by the Brine shrimp lethality assay according to the procedure of McLaughlin *et al.* with slight modifications. The extracts were evaporated, freeze dried and stored until use. The lyophilisates were then reconstituted with DMSO (dimethylsulfoxide) and stock solutions were prepared. After 24 hours of exposure, the percentage of mortality of the *Artemia* larvae was evaluated and the LC₅₀ values were calculated using probit regression analysis. The selected plant extracts were classified according to Meyer's scale and Clarkson's toxicity scale.

RESULTS: The antioxidant activity for the selected samples decreases in the following order: *Origanum majorana* (0.24 mg/mL) > *Carthamus tinctorius* (2.69 mg/mL) > *Sinapis alba* (7.02 mg/mL). Furthermore, a positive correlation between the total phenolic content/total flavonoid content and the antioxidant activity of the ethanol extracts was detected. Highest total phenolic content (340.501 mg/g) and total flavonoid content (90.333 mg/g) showed the extract of *Origanum majorana*. The cytotoxic potential of the spices decreases in the following manner: *Carthamus tinctorius* (20 µg/mL) > *Sinapis alba* (21 µg/mL) > *Origanum majorana* (22 µg/mL). According to Meyer's scale of toxicity, all tested samples were classified as toxic, while Clarkson's scale classified all extracts as highly toxic.

CONCLUSIONS: Although, DPPH assay and Brine shrimp lethality assay do not determine which compounds are responsible for the antioxidant and toxic effects, respectively, they are still very useful methods for the selection of herbal products as promising natural antioxidant and cytotoxic resources. It is beneficial to use the plant antioxidants in the prevention of pathological disorders caused by free radicals. Further studies are needed to demonstrate the correlation between the antioxidant activity and cytotoxic potential of the studied spices.

ESSENTIAL MINERALS IN PORTUGUESE EDIBLE SEAWEEDS

Cristina Soares¹, Simone Morais¹, Susana Machado¹, Ana Carvalho¹,
Manuela Correia¹, Maria João Ramalhosa¹, Teresa Oliva-Teles¹,
Valentina F. Domingues¹, Filipa Antunes², Teresa A.C. Oliveira²,
Cristina Delerue-Matos¹

¹ REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, ISEP, Instituto Politécnico do Porto, Porto, Portugal,

² WEDOTECH, Porto, Portugal.

*Corresponding author: cmm@isep.ipp.pt

KEYWORDS: Seaweeds, essential minerals, macronutrients, micronutrients.

INTRODUCTION: Diets that include marine food on a regular basis present some health benefits regarding nutritionally relevant compounds such as minerals, omega-3 fatty acids and vitamin E. Seaweeds are interesting marine organisms due to their nutritional, commercial and ecological importance. Seaweeds may play a particular role in human nutrition, bridging mineral deficiencies, mainly because their mineral content is ten times as high as that found in plants grown in soil (Mouritsen et al., 2003). The primary mineral components in seaweeds are potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphorous, iodine and chlorine; microelements and trace elements, such as iron (Fe), zinc, copper, manganese, chromium and selenium are also present (Mouritsen et al., 2003). These elements can exist at very different levels depending on the studied species, site of collection and season (Mouritsen et al., 2003).

OBJECTIVES: The main objective of this work is to characterize the mineral content of nine edible seaweeds from the Portuguese coast in order to assess some of their potential health benefits.

METHOD / DESIGN: Seaweeds were collected in coastal waters of the Atlantic Ocean (NW region of Portugal) in April 2016, washed with deionized water and dried in a food dryer (Excalibur 4900 food dryer, Czech Republic). Homogenized samples were microwave-assisted digested based on previous studies (Vieira et al., 2011). After digestion and cooling to ca. 30 °C, samples were kept frozen in polycarbonate containers at -20 °C until analysis. Ca, Mg, Na, K and Fe quantification was carried out using a High-Resolution Continuum Source Flame Atomic Absorption Spectrometer (ContrAA⁺ 700, Analytikjena, Germany) (Torrinha et al., 2014).

RESULTS: The most abundant macronutrients present in the characterized seaweeds are K, Na, Mg and Ca. The median weight content of each metal by species can be represented by K >> Na > Mg > Ca >> Fe for *Sacchorhiza polyschides*; K >> Na > Ca > Mg >> Fe *Laminaria ochroleuca* and *Gracilaria* spp.; Na > K > Mg > Ca > Fe for *Chondrus crispus*, *Mastocarpus stellatus* and *Osmundea pinnatifida*; Na >> K > Mg ≈ Ca > Fe for *Fucus spiralis*, K > Na > Mg > Ca > Fe for *Porphyra* spp. and Mg > Na > K >

Ca > Fe for *Ulva spp.*. This variability may reflect the biological characteristics of each species but also the environmental growing conditions (Mouritsen et al., 2003). Ca levels ranged from 0.99 to 11.25 mg/g of dry seaweed. The highest Ca concentrations were found in brown seaweeds (*S. polyschides*, *F. spiralis* and *L. ochroleuca*). Regarding the essential element Fe, it was detected at concentrations ranging from 0.04 (*O. pinatifida*) to 0.53 (*L. ochroleuca*) mg/g of dry seaweed. Concerning Na/K ratios, values lower than 1 were determined for *Gracilaria sp.*, *Porphyra spp.*, *L. ochroleuca* and *S. polyschides*, while quotients higher than 1 were observed for the other species.

CONCLUSIONS: Seaweeds can contribute to the daily intake of Na, Mg, K, Ca and Fe (0.62% to 5% of the Recommended Daily Allowance/Adequate Intake), based on 1 g of dry seaweed). Also some species present low ratios of Na/K which show the potential for those species to be used as salt substitutes. Overall, the selected species of seaweeds seem to have a potentially interesting nutritional value.

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BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF FRUITS BY-PRODUCTS: EVALUATION OF DIFFERENT EXTRACTION TECHNIQUES

Manuela M. Moreira¹, M. Fátima Barroso¹, Cristina Soares¹, M. J. Ramalhosa¹,
Manuela Correia¹, Aleksandra Cvetanović², Marija Radojković², Jaroslava Svarc-Gajić²
and **Cristina Delerue-Matos**¹

¹ REQUIMTE/LAQV, Instituto Superior de Engenharia do Porto, Rua Dr. António Bernardino de Almeida 431, 4200-072, Porto, Portugal

² Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*Corresponding author: *cmm@isep.ipp.pt*

KEYWORDS: fruits by-products; polyphenols; microwave-assisted extraction; subcritical water extraction; maceration extraction

INTRODUCTION: By-products from the processing of fruits and vegetables, traditionally considered as an environmental problem, are being increasingly recognized as sources for obtaining valuable compounds. Peels, leaves, seeds, stems, and other residues usually contain significant amounts of polyphenols, which are of the utmost interest because of their numerous biological activities [1].

OBJECTIVES: The aim of the present study was to characterize fruits by-products, namely black mulberry leaves, cherry seeds and stems, and pomegranate peels, with a view to exploiting its potential as a source of phenolic compounds.

METHOD / DESIGN: For this purpose, three methods, namely microwave-assisted extraction (MAE), subcritical water extraction (SWE) and maceration extraction (ME), were tested and compared in order to evaluate their efficiency for the recovery of the main polyphenols in these agricultural by-products. Total antioxidant capacity was assessed by means of optical assays, such as total phenolic and flavonoid content, ferric reducing antioxidant power (FRAP), and by the radical scavenging activity-DPPH assay. The polyphenolic profile of these agricultural by-products was evaluated by high performance liquid chromatography with UV detection.

RESULTS: Among the extraction methods evaluated, the highest concentrated extracts were obtained by the MAE technique. Concerning the differences in the fruit by-products analyzed, the extract of pomegranate peels obtained by MAE had the highest concentrations for total phenolic compounds (310.7 ± 26.5 mgGAE/g dry sample) and total flavonoids (44.1 ± 4.2 mgEE/g dry sample), as well as the highest antioxidant activity assessed by FRAP (534.7 ± 34.4 mgAAE/g dry sample) and RSA-DPPH (559.4 ± 60.2 mgTE/g dry sample). On the other hand, the cherry seeds extracts obtained by the ME at 20 °C for 24 h in the dark presented the lowest total phenolic and flavonoid content (6.76 ± 0.56 mgGAE/g dry sample and 1.19 ± 0.11 mgECE/g dry sample, respectively). Characterization of the phenolic composition from the several extracts by HPLC-UV enable to conclude that gallic acid, protocatechuic acid,

β -resorcylic acid, (+)-catechin, and rutin are the main phenolic compounds found in the agricultural by-products analyzed.

CONCLUSIONS: The obtained results evidence the potential of these fruits by-products, namely pomegranate peels, to be used as a source of phenolic compounds, providing a viable alternative both to adding nutritional value to food and to reduce environmental wastes.

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PHENOLIC PROFILE AND BIOLOGICAL ACTIVITY OF CABERNET SAUVIGNON GRAPE JUICE AND WINE

Tatjana Majkić¹, **Diandra Pintać**¹, Ljilja Torović², Dejan Orčić¹, Neda Mimica-Dukić¹, Marija Lesjak¹, Ivana Beara¹

¹ *University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia,*

² *Institute of Public Health of Vojvodina, Futoška 121, 21000 Novi Sad, Serbia*

*Corresponding author: diandra.pintać@dh.uns.ac.rs

KEYWORDS: Cabernet Sauvignon; phenolic profile; antioxidant; acetylcholinesterase

INTRODUCTION: Health benefits of wine, particularly red wine, are recognized worldwide by “French Paradox”, which states that a moderate daily consumption of red wines contribute to lower incidence of coronary heart disease in France despite high levels of saturated fats in the traditional French diet. Cabernet Sauvignon is one of the most common and widespread grape varieties used for wine production. Despite extensive consumption, there is little data about chemical composition and biological activity of this variety grown in Serbia.

OBJECTIVES: In this study, grape juice (juice obtained by immediate pressing of crushed grapes), young wine (first wine obtained after fermentation) and wine from Cabernet Sauvignon, grown on Fruška Gora vineyards, Serbia, were evaluated by in-depth polyphenolic profile characterization and determination of biological activities, including antioxidant and neuroprotective.

METHOD / DESIGN: In order to thoroughly evaluate phenolic profile of investigated wine samples, quantitative analysis of 47 phenolic compounds was performed using the LC-MS/MS technique, while quantification of 5 anthocyanin glucosides was done by LC-UV/VIS technique. The antioxidant potential of samples was determined using several assays: DPPH• and •NO scavenging ability, Ferric Reducing Antioxidant Power (FRAP) and lipid peroxidation inhibition assays. Neuroprotective effect was estimated through potential to inhibit acetylcholinesterase (AChE), an enzyme deeply involved in pathogenesis of neurological diseases, such as Alzheimer’s.

RESULTS: Among examined phenolic compounds, the epicatechin was dominant flavonoid in Cabernet Sauvignon juice, young wine and wine (6.45, 15.5 and 10.8 mg/L, respectively), while the ellagic acid was the most abundant phenolic acid (1.03, 1.98, 8.99 mg/L, respectively). Among anthocyanins, malvidin-3-O-glucoside was the dominant in all samples (110, 160, 3.8 mg/L, respectively). Considering biological potential, wine sample exhibited the highest neuroprotective activity, as well as antioxidant activity in all assays, except in DPPH test, in which young wine showed the highest potential. In comparison to propyl gallate, a well known synthetic antioxidant, and galantamine, a standard inhibitor of AChE, the samples showed moderate activity.

CONCLUSIONS: This study reports novel and valuable data about phenolic profile and biological activity of juice and wine obtained from Cabernet Sauvignon grapes grown in Serbia. Moreover, obtained results implicate that examined grape products have potential to be regarded as promising source of natural antioxidants and neuroprotective agents.

DETERMINATION OF TOTAL POLYPHENOL CONTENT IN AQUEOUS FRUIT EXTRACT OF RUMEX CRISPUS L., POLYGONACEAE

Dunja Jakovljević¹, Tatjana Ćebović¹, Zoran Maksimović², Dragana Četojević-Simin³

¹ Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia,

² Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia,

³ Faculty of Medicine, Oncology Institute of Vojvodina, Dr Golmana 4, Sremska Kamenica, Serbia

*Corresponding author: dunja.jakovljevic@yahoo.com

KEYWORDS: *Rumex crispus*; yellow dock; extract; flavonoids; phenolics.

INTRODUCTION: Apart from being considered a seriously invasive weed, young leaves of yellow dock (*Rumex crispus* L.) are edible and often used as vegetables or salad. The use of its fruits has been described in Serbian and Turkish traditional medicine against stomach complaints. Furthermore, fruits extract of *Rumex crispus* has showed antitumor activity against several human malignant- transformed cell lines.

OBJECTIVES: The objective of this study was to selectively extract several classes of compounds present in the fruits of *Rumex crispus*, and to identify them by High-performance liquid chromatography (HPLC). All fractions were analysed by MS and compared to databases.

METHOD / DESIGN: Ripe yellow dock fruits were collected during summer from a meadow at Kumodraž, the suburbs of Belgrade. The plant material was reduced to a fine powder and extracted with water. The aqueous extract was evaporated until a deep brownish-red dry powder had been produced.

Purification of anthocyanin-containing extracts is often necessary, as the solvent systems commonly used for extraction are not specific for anthocyanins. Anthocyanin purification using solid-phase extraction permits the removal of several interfering compounds present in the crude extracts. Mini-columns containing C18 chains bonded on silica retain hydrophobic organic compounds (anthocyanins and phenolics), while allowing matrix interferences such as sugars and acids to pass through to waste. Washing the retained pigments with ethyl acetate will further remove phenolic compounds other than anthocyanins. The phenolic compounds were analyzed by High-performance liquid chromatography (HPLC). After subjecting the extract to column chromatography, all fractions were analysed by MS and compared to databases.

RESULTS: Major constituents: gallic acid, catechin, hyperoside and miquelianin appear in aqueous fruits extract of yellow dock in concentration 0.35%, 0.048%, 0.79% and 2.45%, respectively.

CONCLUSIONS: By this study we showed that *Rumex crispus* aqueous fruits extract contains flavonoids, as substances which might have biological activity that should be investigated in further studies.

COULD CONIFERS DIMINISH DAMAGES CAUSED BY OXYGEN RADICALS

Dubravka Štajner¹, Boris M. Popović¹, Ružica Ždero Pavlović¹,
Bojana Blagojević¹, Saša Orlović²

¹University of Novi Sad, Faculty of Agriculture, ²University of Novi Sad, Institute of Lowland Forestry and Environment

* Corresponding author: stajnerd@polj.uns.ac.rs

KEYWORDS: antioxidant; conifers; scavenging capacity;

INTRODUCTION: Most of conifer trees are medicinal plants. Seeds, dried needles and fruits of conifers are used to treat various diseases such as bronchitis, common cold, inflammation, rheumatic symptoms and others. Medicinal use of conifers, which are rich in phytochemicals with antioxidant properties, can reduce the risk of human disease such as heart disease, diabetes mellitus, rheumatic disease, liver disorders, multiple sclerosis, Parkinson's disease, autoimmune diseases, Alzheimer's, carcinogenesis etc.

OBJECTIVES: This work highlights an antioxidant capacity of Serbian conifer plants.

METHOD / DESIGN: Antioxidant activities of the crude needles extracts were assessed using different assays. Quantities of phenolic compounds (total phenols, flavonoids, tannins and proanthocyanidins), contents of photosynthetic pigments (chlorophyll a and b and carotenoids), soluble proteins and proline were examined. MDA content, antiradical power toward OH, NO, and O₂⁻ were also investigated. Furthermore, antioxidant activities of extracts against DPPH, ferric reducing antioxidant power and permanganate reducing antioxidant capacity were also determined.

RESULTS: According to almost all used assays, antioxidant and scavenging capacities of fir (*Abies alba* Mill.), and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) were superior compared to spruce (*Picea abies* (L.) H. Karst). Presented results demonstrated that needles of Douglas fir and fir possess outstanding antioxidant capacity, that could reduce damage caused by oxygen radicals responsible for many of the physiological changes and susceptibility to different diseases.

CONCLUSIONS: Presented results implicate that needles of Douglas fir and fir possessed outstanding antioxidant characteristics that could reduce damage caused by oxygen radicals. The presented data provide foundation for extended use of conifers biodiversity in health preservation and in producing remedies for preventing and curing some "free radicals diseases". Investigated conifer plants with high antioxidant activity could increase the resistance toward oxidative damages and they may have a substantial impact on human health that should be proven *in vivo* studies.

CELL-DEATH MECHANISM OF *RUMEX CRISPUS*

L. FRUIT AQUEOUS EXTRACT

Dunja Jakovljević¹, Dragana Četojević-Simin², Tatjana Čebović¹, Zoran Maksimović³

¹ Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia,

² Faculty of Medicine, Oncology Institute of Vojvodina, Dr Golmana 4, Sremska Kamenica, Serbia,

³ Faculty of Pharmacy, Vojvode Stepe 450, Belgrade, Serbia

* Corresponding author: dunja.jakovljevic@yahoo.com

KEYWORDS: *Rumex crispus*; yellow dock; extract; cell death; ELISA

INTRODUCTION: Regarding biological effects, the roots, leaves and fruits of yellow dock (*Rumex crispus* L., Polygonaceae) have been used in traditional medicine for many centuries as a tonic, laxative, spasmolytic and cholagogue agent in bilious complaints, astringent for hemorrhoids and bleeding and for stomach disorders. Due to their edibility, the young leaves of yellow dock are also used as vegetables or salad. Recently, it has been proven that aqueous extract of *R. crispus* possess high anti-tumor activity *in vitro*.

OBJECTIVES: The purpose of this study was determination of *in vitro* mechanism of cell-death induced by *Rumex crispus* fruits aqueous extract in human tumor cell lines.

METHOD / DESIGN: Ripe yellow dock fruits were collected during summer of 2013 from a meadow in Kumodraž (Belgrade, Serbia). The plant material was reduced to a fine powder, extracted with water and evaporated. The mechanism of cell-death in human cervix carcinoma (HeLa) and breast adenocarcinoma (MCF7) cell lines was determined by detection of apoptosis and necrosis using Cell Death Detection Elisa Plus kit. This enzyme-immunoassay qualitatively and quantitatively determines cytoplasmic histone associated DNA fragments after induced cell death.

RESULTS: *Rumex crispus* extract slightly decreased apoptosis and significantly increased necrosis in both MCF7 (EFA=0.77; EFN=2) and HeLa (EFA=0.97; EFN=4) cell lines giving high overall decrease in apoptosis/necrosis ratios compared to control (EF A/N= 0.24 - 0.39). Analysis of cytotoxicity mechanism showed that the necrosis was the main mechanism of induced cell death in cervix (HeLa) and breast (MCF7) tumor cell lines after treatment with *Rumex crispus* fruits water extract.

CONCLUSIONS: It can be concluded that fruit extract of *Rumex crispus* has high cytotoxic activity, with necrosis as a main mechanism of induced cell death. Different method of extraction of *Rumex crispus* fruits, apart from aqueous, is recommended in order to pinpoint possible active principles with lower necrotic and higher apoptotic potential that will also retain high antitumor potential.

BIOLOGICAL POTENTIAL OF SPECIES FROM THE GENUS *SALIX*

Emilia Šefer¹, Nevena Grujić-Letić¹

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacy, HajdukVeljkova 3, 21000 Novi Sad, Serbia

* Corresponding author: emilia.sefer@mf.uns.ac.rs

KEYWORDS: *Salix*; antioxidant activity; DPPH; OH

INTRODUCTION: Since ancient times willow bark (*Salix sp.*, Salicaceae) has been used to treat pain, fever and inflammation. Its anti-inflammatory effect is closely related to the antioxidant activity of phenolic compounds. The main biological source of willow bark is *Salix alba*, but other species of the genus *Salix* are often used as substitute in herbal preparations. Many species of willow, widely distributed in nature, are poorly studied until present. Moreover, the data on medicinal features of leaves of *Salix* species are lacking.

OBJECTIVES: The objective of this study was to compare the antioxidant potential and total phenolic and flavonoid content of bark and leaf of four species of the genus *Salix*: *S. alba*, *S. babylonica*, *S. elaeagnos* and *S. fragilis*.

METHOD / DESIGN: Bark and leaf extracts were prepared by maceration with 70% (v/v) ethanol for 48 hours. Antioxidant activity of extracts was assessed by measuring their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl (OH) radicals. Total phenolic content was determined according to Folin-Ciocalteu procedure, while flavonoid content was measured by aluminium chloride colorimetric method.

RESULTS: Concentrations of extracts that scavenged 50% of DPPH radicals ranged from 1.83 to 8.07 µg/ml and OH radical from 22.2 to 51.96 µg/ml. *S. alba* bark extract exhibited the strongest DPPH scavenging activity, while *S. fragilis* bark inhibited OH radical the most. Total phenolic and flavonoid contents were in intervals 10.26-61.27 mg GAE/g d.w. and 3.13-23.45 mg QE/g d.w., respectively. The highest amount of total phenolics was found in *S. elaeagnos* bark extract, while the leaf extract of the same species possesses the highest flavonoid content.

CONCLUSIONS: The obtained results indicate that bark and leaf extracts of all investigated species exhibited strong antioxidant activity in both DPPH and OH radical scavenging assays. Bark extracts have higher antioxidant potential than leaf extracts. The amount of total phenolics was higher in bark extracts and total flavonoid content in leaf extracts. The results suggest that barks and leaves of investigated *Salix* species could be used as natural sources of antioxidants.

FUNGAL EXTRACTS AS NEW SOURCE OF ANTIOXIDANT AGENTS

Milena Rašeta¹, **Emilija Svirčev**¹, Maja Karaman², Sanja Vlasisavljević¹,
Mira Popović¹, Neda Mimica-Dukić¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, Novi Sad, Serbia

² University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, Novi Sad, Serbia

*Corresponding author: milena.raseta@dh.uns.ac.rs

KEYWORDS: *Ganoderma*; ABTS^{•+} assay; DPPH[•] assay; [•]OH assay

INTRODUCTION: In the last decades wood decaying fungi of the genus *Ganoderma* (phylum Basidiomycota), have been recognized as important source of pharmacologically active substances. These medicinal fungi species possess various pharmacological effects: antioxidative, antimicrobial, antitumor, cardioprotective, hepatoprotective and immunomodulating.

OBJECTIVES: The aim of this study was to examine antioxidant potency of ethanolic and water extracts of *Ganoderma resinaceum* Boud. In Pat. from Fruška Gora Mountain (Serbia).

METHOD / DESIGN: Antioxidant potency of *G. resinaceum* extracts was evaluated using four *in vitro* assays: AEAC (Ascorbate Equivalent Antioxidant Capacity), ABTS^{•+}, DPPH[•] and [•]OH scavenger capacity assays. Total phenolic and flavonoid content was determined spectrophotometrically.

RESULTS: The highest ABTS^{•+} and [•]OH scavenging activities were found in the water extract, while the highest DPPH[•] activity showed ethanolic extract. Determined antioxidant activities were in good correlation with reducing power assay (AEAC). Total phenolic content was higher in ethanolic extract compared to water extract, while flavonoid content varied slightly between the extracts examined.

CONCLUSIONS: Obtained results indicate that the extracts of these medicinal fungi species are potential source of natural antioxidants, and strongly support use of water extracts of *G. resinaceum* in prevention of free-radical-induced delirious effects on health.

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QUANTIFICATION OF PHENOLIC COMPOUNDS IN *GLYCYRRHIZA GLABRA* L. EXTRACTS

Sanja Vlasisavljević^{1*}, **Filip Šibul**¹, Sanja Vasiljević², Milena Rašeta¹,
Neda Mimica-Dukić

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

² Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

*Corresponding author: filip.sibul@dh.uns.ac.rs

KEYWORDS: *Glycyrrhiza glabra* L.; LC-MS-MS technique; bioactive compounds; phenols

INTRODUCTION: *Glycyrrhiza glabra* L. (licorice) is one of the oldest medicinal plants. It is spread on a wide part of northwest Europe to North Africa, east to Siberia and the Caucasus, as well as in Asia, while it is rarely seen in Serbia. *G. glabra* contains a wide spectrum of bioactive constituents such as triterpens (glycyrrhetic acid, glycyrrhizin), phenols (including liquiritigenin, liquiritin, isoliquiritigenin, isoliquiritin) and many others. These bioactive compounds are potentially beneficial for health because they may protect the body against major degenerative diseases, aging and some cancers.

OBJECTIVES: The present study was undertaken to characterize and quantify the phenolic composition and evaluate the antioxidant activity in fresh and dry root and leaves extracts of *Glycyrrhiza glabra* L. from locality of Fruška Gora, Serbia.

METHOD / DESIGN: Ethylacetate extracts were prepared by microwave-assisted extraction. Dried extracts were dissolved in the mixture of 1% aqueous formic acid to obtain 2% (w/v) stock solutions. The selected phenolic compounds have been quantified using liquid chromatography/tandem-mass spectrometry (LC-MS-MS) technique. The results were expressed as µg/g of dry weight.

RESULTS: Of total forty-five selected phenolic compounds, thirty-one have been quantified. Remarkable differences in quantitative composition of phenolic compounds have been observed. The highest amount of phenolic compounds was found in the extract of fresh *G. glabra* root. The most dominant quantified phenols were quinic acid (730.89 µg/g), naringenin (186.35 µg/g), vitexin (22.88 µg/g), quercetin-3-O-glucoside (29.00 µg/g) and rutin (284.63 µg/g). These compounds are responsible for different biological activities. The obtained results could be explained by the fact that drying process can affect bioactive compounds content and, therefore, their biological activities.

CONCLUSIONS: According to the obtained results, it can be concluded that extract of fresh root of *G. glabra* from Serbia is a valuable source of natural antioxidants, which could indicate their use as possible agents against prevention of oxidative stress and thereby reduce the development of many diseases.

METHOD FOR SIMULTANEOUS LC-MS/MS QUANTIFICATION OF 25 COMPOUNDS COMMONLY OCCURRING IN PLANTS

Filip Šibul¹, Dejan Orčić¹, Sanja Berić¹, Nataša Simin¹, Emilija Svirčev,¹ Marina Francišković¹, Neda Mimica-Dukić¹

¹ *University of Novi Sad, Faculty of Sciences, Department for Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*

*Corresponding author: filip.sibul@dh.uns.ac.rs

KEYWORDS: quantification; plant compounds; LC-MS/MS

INTRODUCTION: Plants contain a complex mixture of secondary metabolites - compounds produced throughout their growth and different stages of vegetation. In order to understand the effects of plants commonly used as traditional remedies, it is important to get an insight into their chemical composition and concentrations of the present compounds. For identification and quantification of most of the water-soluble, polar and medium-polar compounds present in plants, reversed phase liquid chromatography coupled with triple quadrupole mass spectrometry is a quantification technique of choice.

OBJECTIVES: Since there is a growing interest in pharmacological effects of plants, development of rapid LC-MS/MS methods is therefore needed for fast quantitative determination of plant compounds. The method for simultaneous quantification of 25 compounds commonly occurring in plants is thus hereby developed.

METHOD / DESIGN: All extracts were diluted in mixture of water and methanol premixed in 1:1 ratio, to obtain a final concentration 2 mg/mL. Fifteen working standards, ranging from 1.53 ng/mL to 25.0 µg/mL, were prepared and all samples and standards were analyzed using Agilent Technologies 1200 Series high-performance liquid chromatograph coupled with Agilent Technologies 6410A Triple Quad tandem mass spectrometer with electrospray ion source, and controlled by Agilent Technologies MassHunter Workstation software - Data Acquisition (ver. B.03.01). Compounds were separated on Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 µm) rapid resolution column held at 50 °C. Mobile phase consisted of A: 0.05% aqueous formic acid and B: methanol, and was delivered at flow rate of 1 mL/min in gradient mode (0 min 30% B, 6 min 70% B, 9 min 100% B, 12 min 100% B, re-equilibration time 3 min). Eluted compounds were detected by ESI-MS/MS, using the ion source parameters as follows: nebulization gas (N₂) pressure 50 psi, drying gas (N₂) flow 10 L/min and temperature 350 °C, capillary voltage 4 kV, negative polarity. Data were acquired in dynamic MRM mode, using the optimized compound-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage). 25 compounds are chosen to be included into the method, based on their occurrence in plants and availability of reference standards: 10 flavonoids (pinocembrin, pinostrobin, hesperetin, liquiritigenin, naringin, chrysin, galangin, morin, rhamnetin, diosmetin), 5 anthraquinones

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(dantron, alizarin, rhein, emodin, aloe-emodin), *cis,trans*-abscisic acid, isoliquiritigenin, elagic acid, glycyrrhizin, 18 β -glycyrrhetic acid, 4-hydroxyphenylacetic acid, formononetin, isoscapoletin, ursolic acid and resveratrol.

RESULTS: Retention times (t_R) showed good repeatability, with standard deviations no greater than 0.004 min for standards in solvent, and no greater than 0.146 min for the spiked matrix. During a calibration study, a slight deviation from linearity was observed at high concentrations. Therefore, the limit of linear range was determined as the highest standard concentration still resulting in satisfactory linearity ($r^2 > 0.98$) of weighted calibration curve, for each compound. A reliable quantitation limit (LoQ) was estimated at the lowest concentration still resulting in an acceptable repeatability (<10%). Limit of detection (LoD) was estimated as the lowest concentration resulting in well-defined peak. The procedure was repeated in matrix. *M. chamomilla* flos (100 μ g/mL) was spiked with a standard mixture and reanalysed in 5 replicates, which resulted in only one peak being observable at specified retention time, for each compound. The peak width at half-maximum in spiked samples was in range 94–108% of peak width in non-spiked sample, which is within acceptable limits as defined in European Community Council Directive 96/23/EC. The retention times of all investigated compounds differed in spiked and non-spiked samples by –0.5% to 0.5%, which is also within the prescribed margin (5%). Matrix effects were quantified by use of postextraction addition method. Solvent calibration (SC) and matrix-matched calibration (MMC) standards were prepared at all levels for all investigated compounds. For each compound, signal suppression/enhancement (SSE) was calculated as ratio of responses of a compound in matrix and in solvent. For majority of investigated compounds, SSE was close to 1 (with deviation less than ± 0.25) in a wide concentration range.

CONCLUSIONS: The dynamic MRM LC-MS/MS method for simultaneous quantification of 25 compounds commonly occurring in plants was hereby successfully developed and validated as a valuable tool for future determinations of plant extracts' chemical composition.

ANTHOCYANIN COMPOSITION OF WINES FROM FRUŠKA GORA REGION (SERBIA)

Ljilja Torović¹, Marija Lesjak², Tatjana Majkić², Diandra Pintac², Neda Mimica-Dukić², **Ivana Beara**²

¹ *Institute of Public Health of Vojvodina, Futoška 121, 21000 Novi Sad, Serbia*

² *University of Novi Sad Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia*

*Corresponding author: ivana.beara@dh.uns.ac.rs

KEYWORDS: anthocyanins; wine; Fruška Gora

INTRODUCTION: Anthocyanins are polyphenols of the flavonoid family. In *Vitis vinifera* L. the monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin as well as their acetyl-, *p*-coumaroyl- and caffeoyl derivatives are present in quantities varying among varieties, climate and viticulture conditions. Anthocyanins play a key role in the colour characteristics and colour stability of wines. At winemaking, they are partially extracted from grape skins to the must, becoming responsible for the color of young red wine. During maturation and aging of wine, levels of anthocyanins decrease with time, since they begin to react with other wine constituents leading to the formation of more stable pigments that are responsible for colour changes. Anthocyanins are the major polyphenolics in red wines. Due to their antioxidant and free radical scavenging activities, polyphenols are considered to be principally responsible for health-protective benefits of moderate wine consumption, most importantly – prevention of cardiovascular diseases.

OBJECTIVES: The objective of this research was to study and compare anthocyanin composition of red wines produced from five grape varieties: Merlot, Cabernet Sauvignon, Pinot Noir, Portuguiser and Frankovka, in wineries on Fruška Gora (Serbia).

METHOD / DESIGN: A set of 28 monovarietal red wines of vintages from 2009 to 2015, produced and bottled in local wineries on Fruška Gora, collected either directly from the producers or from the local market, included 10 samples of Merlot, six of Cabernet Sauvignon, five of Pinot Noir, four of Portuguiser and three of Frankovka. Samples were subjected to the analysis of five major anthocyanins by high performance liquid chromatography, including separation on a reverse phase Poroshell 120 EC-C18 column (4.6 × 100 mm, 2.7 μm; Agilent), heated at 40 °C, with gradient elution by water/formic acid/acetonitrile mixtures (A – 87:10:3, B – 40:10:50; flow rate 0.8 mL/min; run time 14 min) and UV-Vis detection at 518 nm.

RESULTS: Concentrations of individual anthocyanins, 3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, were determined in wine samples. Furthermore, the average concentrations of total anthocyanins grouped according to variety were calculated, giving the following results: Frankovka 92 mg/L, Portuguiser 54 mg/L, Merlot 39 mg/L, Cabernet Sauvignon 28 mg/L and Pinot Noir 11 mg/L.

As expected, generally higher content of monomeric anthocyanins was found in the younger wines, regardless of the variety. Taking into account that sample collection was not representative for every variety for all vintages, obtained data were not appropriate for ranking of varieties according to the anthocyanin content. In order to facilitate the comparison of anthocyanin profiles of wines, the individual compound concentrations were expressed as percent of total anthocyanins. Data analysis pointed to malvidin-3-*O*-glucoside as the predominant anthocyanin in the wines of all studied grape varieties. The proportion of malvidin-3-*O*-glucoside in Merlot wines (77%) was lower compared to Frankovka (86%), Cabernet Sauvignon (83%), Portuguiser and Pinot Noir (85%) wines. The contribution of the four remaining 3-*O*-glucosides decreased in the following order: peonidin > petunidin > delphinidin > cyanidin.

CONCLUSIONS: The reported results represent an input to the database of the anthocyanins occurrence in red wines from the most common grape varieties grown on Fruška Gora. In depth chemical characterization should be regarded as a valuable foundation to all wine entrepreneurs in valorisation, positioning and strengthening the recognition and excellence of wines from this important wine region of Serbia.

**BIOLOGICAL POTENTIAL OF 16-OXIMINO
DEHYDROEPIANDROSTERONE DERIVATIVES**

Ivana Kuzminac¹, Andrea Nikolić¹, Marina Savić¹, Jovana Ajduković¹,
Edward Petri², Vesna Kojić³, Marija Sakač¹

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences,
University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia,

² Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja
Obradovića 4, 21000 Novi Sad, Serbia,

³ Oncology Institute of Vojvodina, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia

*Corresponding author: ivana.kuzminac@dh.uns.ac.rs

KEYWORDS: steroids; oximes; antiproliferative activity; drug-likeness; Lipinski and
Veber rules

INTRODUCTION: Chemically modified steroids have found significant application
in hormone dependent cancer treatment. 17-Keto-16-oximino steroid derivative **1**
and its reduction product **2** have been used for synthesis of series of steroidal com-
pounds that have shown significant biological potential. Unfortunately, these oximes
have never been tested for their drug-likeness and anticancer activity.

OBJECTIVES: In this paper we report synthesis of 3 β -hydroxyandrost-5-ene-16,17-
dione 16E-oxime (**1**) and 16E-hydroximinoandrost-5-ene-3 β ,17 β -diol (**2**). These com-
pounds were tested for their cytotoxic activity against six human tumor cell lines and
one healthy human cell line. Furthermore, drug-likeness was analyzed comparing cal-
culated molecular properties with the criteria for Lipinski [1] and Veber [2] rules.

METHOD / DESIGN: 17-Keto-16-oximino steroid derivative **1** and its reduction
product **2** were obtained from dehydroepiandrosterone according to a known proce-
dure [3]. Antiproliferative activity of compounds **1** and **2** was tested against a panel of
human cancer cell lines: MCF-7 (human breast adenocarcinoma, ER+), MDA-MB-231
(human breast adenocarcinoma, ER, triple-negative), PC3 (prostate cancer), HeLa
(human cervical carcinoma), HT-29 (colon cancer) and A549 (human lung carcino-
ma), using the standard MTT assay. Normal, non-cancerous cells (MRC-5 fetal lung
fibroblasts) were used as a cytotoxicity control. Results were analyzed with respect to
a nonselective cytotoxic drug (cisplatin) and the steroidal CYP19/aromatase inhibitor
formestane. Molecular properties that influence the oral bioavailability of drug candi-
dates were computed employing the Molinspiration online property calculation toolkit
and compared with Lipinski and Veber rules of drug-likeness.

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RESULTS: Table 1. Cytotoxic activity of compounds **1** and **2**

Comp.	IC ₅₀ (μM)						
	MCF-7	MDA-MB-231	PC-3	HeLa	HT-29	A549	MRC-5
1	90.56	8.62	>100	>100	>100	58.77	>100
2	10.18	5.57	63.79	>100	>100	15.78	>100
cisplatin	1.60	2.64	4.56	2.10	4.10	3.20	0.24
formestane	>100	19.61	26.37	3.36	>100	38.59	>100

Table 2. Calculated molecular properties

Comp.	MW	miLogP	HBA	HBD	Lipinski violations	TPSA	nrotb	Veber violations
1	317.43	2.59	4	2	0	69.89	0	0
2	319.44	2.78	4	3	0	73.05	0	0

MW, molecular weight (g mol⁻¹); logP, logarithm of compound partition coefficient between n-octanol and water; HBA, number of hydrogen bond donors; HBD, number of hydrogen bond acceptors; TPSA, topological polar surface area (Å²); nrotb, number of rotatable bonds.

CONCLUSIONS: In cytotoxicity test, oximino ketone **1** has shown high selectivity towards ER-human breast adenocarcinoma, while oximino alcohol **2** has demonstrated potent cytotoxicity towards three neoplastic cell lines. These compounds have not shown any violation of Lipinski and Veber rules of drug-likeness. Obtained results indicate that further biological tests should be performed.

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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SOME NEW A-RING FUSED PYRIDINE D-MODIFIED ANDROSTANE DERIVATIVES

Marina Savić¹, Jovana Ajduković¹, **Ivana Kuzminac**¹,
Dimitar Jakimov², Evgenija Djurendić¹

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

² Oncology Institute of Vojvodina, Put Dr Goldmana 4, 21204 Sremska Kamenica, Serbia

*Corresponding author: marina.savic@dh.uns.ac.rs

KEYWORDS: synthesis; pyridine androstane derivatives; antiproliferative activity

INTRODUCTION: In recent years researchers are directed toward the synthesis of androgen hormones, proving their structures and their application for therapeutic purposes. Namely, it has been shown that certain androgenic hormones are responsible for the occurrence of prostate cancer, benign prostatic hyperplasia, brain cancer, carcinoma of the ovary, and breast cancer. An extensive focus of research is directed towards the rational modification of steroid molecules. Pyridine ring condensed with steroidal nucleus was found to be a structural element that contributes to the biological activity.

OBJECTIVES: Three series of steroidal derivatives with A-ring fused pyridine moiety were synthesized and their antiproliferative activity was evaluated. These new compounds were synthesized in D-homo lactone, 17 α -picolyl and 17(*E*)-picolinylidene series and they were screened for antiproliferative activity against six malignant human cell lines (human breast adenocarcinoma ER+, MCF-7, human breast adenocarcinoma ER-, MDA-MB-231, prostate cancer AR-, PC-3, cervical cancer, HeLa, colon cancer, HT-29 and lung adenocarcinoma, A549), as well as healthy fetal lung fibroblasts (MRC-5).

METHOD / DESIGN: This work represents a continuation of our previous work, where the new androstane derivatives with pyridine ring fused to the 3,4-position of the steroid nucleus were obtained. These new pyridine androstane derivatives were prepared in one-pot reaction of the starting compounds with propargylamine catalyzed by Cu(II). Synthesized compounds were evaluated for antiproliferative activity against six malignant cell lines, as well as control non-cancerous cell line *in vitro*, after 48 h treatment using MTT assay. Nonselective drug doxorubicin and steroidal aromatase inhibitor formestane were used as a positive control.

RESULTS: To determine the effect of pyridine ring condensed with steroidal nucleus on antiproliferative activity of parental molecules, we have synthesized four new compounds **1-4** (Figure 1.). Pyridino[2,3':3,4]-17 α -picolyl-androst-5-en-17 β -ol (**1**), pyridino[2,3':3,4]-17(*E*)-picolinyliden-androst-5-ene (**2**), pyridino[2',3':3,4]-17-oxa-17a-homoandrostan-16-one (**3**) and pyridino[2',3':3,4]-17-oxa-17a-homoandrostane-6,16-dione (**4**) were obtained by reactions of corresponding starting compounds

with propargylamine in ethanol under reflux and argon atmosphere, catalyzed by copper(II)-nitrate trihydrate, yielding 63%, 50%, 30% and 16%, respectively. New compounds **1-4** were evaluated for antiproliferative activity and results are given in Table 1.

Table 1. IC₅₀ values of the tested steroidal and referent compounds

Compound	IC ₅₀ (μM)						
	MCF-7	MDA-MB-231	PC-3	HeLa	HT-29	A549	MRC-5
1	>100	44.52	7.93	>100	46.08	>100	>100
2	37.42	87.37	11.47	>100	>100	11.89	>100
3	>100	9.13	>100	11.77	>100	>100	>100
4	>100	>100	>100	>100	0.06	>100	>100
formestane	>100	55.5	48.36	5.55	>100	>100	>100
doxorubicin	0.75	0.12	95.61	1.17	0.32	0.36	0.12

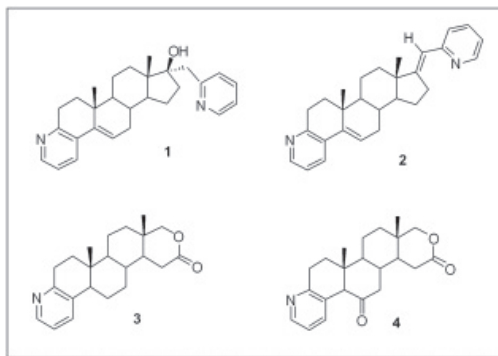


Figure 1. Molecular structure of new A-ring fused pyridine D-modified androstane derivatives

CONCLUSIONS: It can be observed that compounds showed antiproliferative activity against the tested cell lines. Compound **3** exhibited strong antiproliferative activity against MDA-MB-231 and HeLa cells, while compounds **1** and **2** exhibited strong antiproliferative activity against PC-3 cells. Also, compound **2** showed strong antiproliferative activity against A549 cells. Compound **4** is the most promising compound, with submicromolar activity against HT-29 cells, which is about 5-fold higher in compare with commercial cytostatic doxorubicin. It is important to emphasize that all four compounds were nontoxic to healthy MRC-5 cells.

**CARBAMATES AND CYCLIC UREAS AS INHIBITORS OF α -GLUCOSIDASE:
IN VITRO ACTIVITY TESTING AND QSAR STUDY**

Ivana Jevtić¹, Jelena Popović-Đorđević², Nađa Grozdanić Stanislavljević³,
Sandra Šegan⁴, Mario Zlatović¹, Milovan Ivanović¹, Tatjana Stanojković³

¹ Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

² Faculty of Agriculture, Chair of Chemistry and Biochemistry, University of Belgrade, Belgrade, Serbia

³ Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

⁴ Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

*Corresponding author: jelenadj@agrif.bg.ac.rs

KEYWORDS: α -glucosidase inhibitors; carbamates; cyclic ureas; QSAR

INTRODUCTION: Diabetes mellitus type 2 (T2DM) is an endocrine disease of global proportions which is currently affecting 1 out of 12 adults in the world, still increasing prevalence. The broad range of oral antidiabetic drugs is available today for T2DM. The main classes vary in their chemical composition, modes of action, safety profiles and tolerability. Some of them like sulphonylureas act as insulin secretion stimulators, while α -glucosidase (α -Gls) inhibitors postpone digestion and absorption of intestinal carbohydrate. Glycosidase enzymes catalyze the hydrolysis of glycosidic linkages by releasing monosaccharides from the non-reducing end of an oligosaccharide or glycoconjugate, and are implicated in an array of vital biological processes. During the recent years α -Gls inhibitors have been the subject of various studies in the field of medicinal chemistry. Since 1990s some of carbohydrate derivatives like acarbose (Glucobay[®], Precose[®], Prandase[®]), voglibose (Basen[®]), and miglitol (Glyset[®]) are used as α -Gls inhibitors for the treatment of diabetes. Nevertheless, complicated multi-step synthetic procedures imposed a need for a synthesis of compounds with no structural similarity to the above compounds.

OBJECTIVES: The aim of the study was to investigate the inhibitory activity of five cyclic urea derivatives and eight carbamates to α -glucosidase (α -Gls) in *in vitro* assay. In order to rationalize structural features associated with the most potent compounds quantitative structure-activity relationship (QSAR) was done.

METHOD / DESIGN: Anti α -glucosidase assay was performed using colorimetric enzyme test. A series of 13 compounds dilutions were let to inhibit enzyme activity for 15 minutes, before substrate 4-nitrophenyl α -D-glucopyranoside was added. Absorbances were read at 405 nm, and results analysed to get IC₅₀ value for each compound and acarbose (as a positive control). All tests were done in duplicate.

Structure building, calculation of physically significant molecular descriptors and pharmaceutically relevant properties and SE calculations using the RM1 method were performed using various modules from Schrodinger Suite 2015-1. PLS has been per-

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formed using a demo version of PLS_Toolbox statistical package (Eigenvectors, v. 5.7) for MATLAB version 7.4.0.287 (R2007a).

RESULTS: The anti α -glucosidase activity study showed that tested compounds expressed significant activity. All 13 compounds showed better anti α -glucosidase activity than standard antidiabetic drug acarbose, with IC_{50} ranged from $49.85 \pm 0.1 \mu\text{M}$ for benzyl(3,4,5-trimethoxyphenyl) carbamate to $104.06 \pm 0.65 \mu\text{M}$ for *cis*-5-phenethyl-1-phenylhexahydro-1H-imidazo[4,5-c]pyridin-2(3H)-one. IC_{50} for acarbose was $121.01 \pm 12.18 \mu\text{M}$. Cyclic ureas and carbamates (**1-13**) were synthesized in high yields from easily obtained carboxamide precursors, by modified Hofmann rearrangement. We tried to relate molecular descriptors of the compounds (constitutional, topological, geometrical, electrostatic, QM characteristics), with their biological activities using QSAR. Results showed that the most relevant predicted descriptors were: conformation-independent aqueous solubility (CIQPlogS), hydrophilic component of the total solvent accessible surface area (FISA), Percent human oral absorption, skin permeability (QPlogKp), octanol/water partition coefficient (QPlogPo/w), ionization potential (IP), apparent MDCK cell permeability (QPPMDCK). The interpretation of descriptors included in PLS model can help us in better understanding and explanation of biological behavior of investigated compounds.

CONCLUSIONS: Cyclic ureas and carbamates showed promising anti α -glucosidase activity and should be tested further as potential antidiabetic drugs. The results of QSAR study can indicate the descriptors that play the important role in the activity exhibited by similar compounds, and thus can help us planning the future synthesis of more potent compounds.

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CARDIOBUTANOLIDE ANALOGUES AS ANTITUMOR AGENTS: SYNTHESIS AND BIOLOGICAL EVALUATION

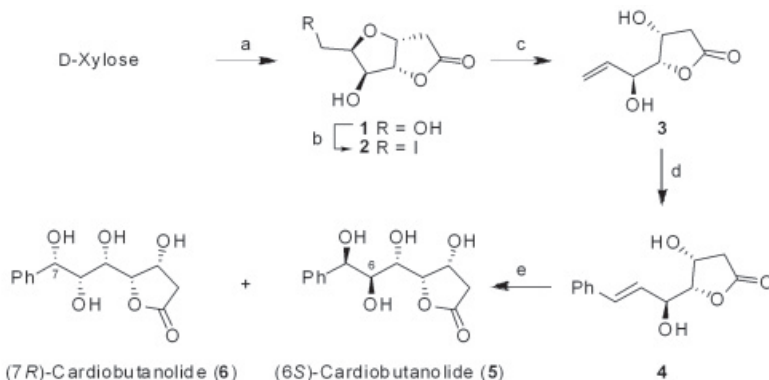
Ivana Kovačević¹, Mirjana Popsavin¹, Goran Benedeković¹, Jovana Francuz¹,
Bojana Srećo Zelenović¹, Miloš Svirčev¹, Marko Rodić¹,
Vesna Kojić², Velimir Popsavin¹

¹ Faculty of Sciences, University of Novi Sad, Trg D. Obradovića 3, Novi Sad, ²Oncology Institute of Vojvodina, Put Dr. Goldmana 4, Sremska Kamenica

*Corresponding author: ivana.kovacevic@dh.uns.ac.rs

KEYWORDS: cardiobutanolide; natural product; SAR analysis; antitumor agents; analogue synthesis.

INTRODUCTION: (+)-Cardiobutanolide, naturally occurring styryl lactone isolated from the stem bark of *Goniothalamus cardiopetalus*,¹ as a highly potent antitumor agent, represents a convenient lead for design of novel chemotherapeutics.



Reagents and conditions: (a) Meldrum's acid, Et₃N, DMF, 7 days, 46 °C, 65%. (b) I₂, Im, THF, reflux, 3 h, 63%. (c) activated Zn, THF/H₂O (4:1), 1 h, reflux, 94%. (d) styrene, Grubbs^{II}, atm. Ar, rt, CH₂Cl₂/THF (2:1), 18 h, 50%. (e) 2.5 wt% OsO₄ in ^tBuOH, Me₂CO/H₂O (10:1), rt, 1 h, 16% (5) i 28% (6).

OBJECTIVES: Total synthesis of two novel cardiobutanolide analogues with different stereochemistry at C-7 and C-6 stereocenters. Investigation of their antitumor activity and identification of structural features responsible for their potency, by means of SAR analysis was further important objective of this work.

METHOD / DESIGN: Structure and purity of synthesized compounds was determined by NMR spectroscopy, HRMS and X-ray crystallography. The colorimetric MTT assay was used for the evaluation of antiproliferative activity.

1 Hisham, A.; Toubi, M.; Shualiy, W.; Bai, M. D. A.; Fujimoto, Y. *Phytochemistry* **2003**, 62, 597.
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RESULTS: The most important results are outlined in the scheme and the table below.

Compound	IC ₅₀ (μM), 72 h								
	K562	HL-60	Jurkat	Raji	MCF-7	MDA-MB 231	HeLa	A549	MRC-5
5	30.54	15.61	27.58	14.60	8.05	29.47	2.96	11.85	>100
6	21.54	5.22	8.88	1.95	2.45	35.64	32.58	9.67	>100
DOX	0.25	0.92	0.03	2.98	0.20	0.09	0.07	4.91	0.10

CONCLUSIONS: An efficient, protective group free synthesis of cardiobutanolide analogues **5** and **6** was achieved starting from D-xylose. Preliminary evaluation of antitumor activity showed that potency of synthesized compounds toward selected human tumor cell lines is in the range of 1.95–35.64 μM. Remarkably, these compounds are completely inactive against normal fetal lung fibroblasts (MRC-5).

Acknowledgement: *The work was supported by a grant from the Ministry of Education, Science and Technological Development (Project 172006).*

SYNTHESIS AND EVALUATION OF ANTIPROLIFERATIVE ACTIVITY OF NOVEL GONIOFUFURONE MIMICS

Mirjana Popsavin,¹ **Ivana Kovačević**,¹ Miloš Svirčev,¹ Goran Benedeković,¹ Jovana Francuz,¹ Bojana Srećo Zelenović,¹ Marko Rodić,¹ Vesna Kojić,² Milka Jadranin³ and Velimir Popsavin¹

¹ Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, Trg D. Obradovića 3, Novi Sad,

² Oncology Institute of Vojvodina, Put doktora Goldmana 4, Sremska Kamenica,

³ Faculty of Chemistry, University of Belgrade, Studentski trg 12, Belgrade, Serbia

* Corresponding author: ivana.kovacevic@dh.uns.ac.rs

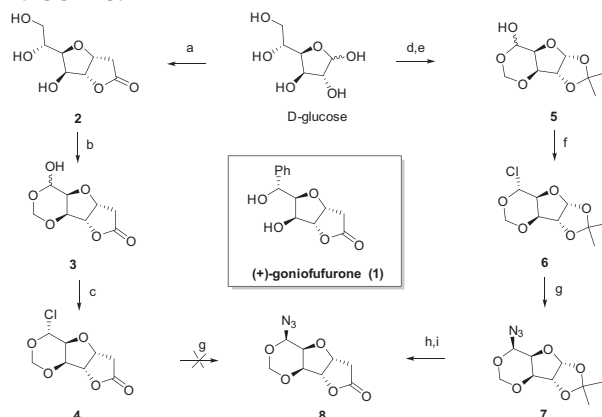
KEYWORDS: goniofufurone; goniofufurone mimics; analogue synthesis; antitumour bioassay; MTT test

INTRODUCTION: Some natural products and their synthetic derivatives are important as drug candidates or lead structures for the discovery of novel drugs. Goniofufurone is naturally occurring γ -lactone that shows remarkable cytotoxicity against certain human neoplastic cells.

OBJECTIVES: In continuation of our ongoing studies on the synthesis of bioactive products from abundant monosaccharides, we have focused on the synthesis of novel tricyclic styryl-lactones due to their potential antitumour activity. Two novel tricyclic goniofufurone mimics (**4** and **8**) were prepared through three- and six-step sequences starting from D-glucose.

METHOD / DESIGN: Methods for checking purity and identity of newly synthesized compounds included ¹H, ¹³C NMR spectra, X-ray structure analysis and high resolution mass spectra. The colorimetric MTT assay was carried out for the evaluation of antiproliferative activity.

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RESULTS:

Scheme 1. Reagents and conditions:

(a) Meldrum's acid, DMF, Et₃N, 66 h, 46–48 °C; (b) NaIO₄, CH₃CN, rt; (c) CCl₄, Ph₃P, anh. CH₂Cl₂, 2,6-lutidine, 24 h, rt; (d) conc. H₂SO₄, Me₂CO, rt; (e) H₃IO₆, EtOAc, rt; (f) MsCl, CH₂Cl₂, Et₃N, 0 °C → +4 °C; (g) NaN₃, DMSO, 1 h, 90 °C; (h) 90% aq TFA, rt, 8 h; (i) Meldrum's acid, DMF, Et₃N, 48 h, 46–48 °C.

CONCLUSIONS: The synthesis of conformationally constrained goniofufurone mimics with 5,7-*O*-methylidene and 7-chloro (compound **4**) or 7-azido functions (compound **8**) is completed by using two independent multi-step sequences, and D-glucose as a starting compound. *In vitro* cytotoxicity of newly synthesized analogues against eleven human tumour cell lines and against a single normal cell line was evaluated and will be presented.

The work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 172006).

DETERMINATION OF TETRAHYDROCANNABINOL IN HAIR SAMPLES BY LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRY

Ivana Bugarski¹, Marko Antunović², Dušica Jovanović³,
Vesna Kilibarda², Snežana Đorđević²

¹ Ministry of Interior of the Republic of Serbia, Police Directorate, Criminalistic Police Department, National Criminalistic-Technical Centre,

² National Poison Control Centre, Military Medical Academy, Belgrade, ³ Faculty of Physical Chemistry, University of Belgrade

*Corresponding author: ivanabugarski@gmail.com

KEYWORDS: Cannabis; THC; LC-MS; hair analysis.

INTRODUCTION: Cannabis is the most broadly produced illicit drug in almost every country worldwide, and Cannabis usage shows highest prevalence among illicit drugs globally. Cannabinoid analysis in hair samples is conducted in order to identify psychoactive Δ^9 -tetrahydrocannabinol (THC), cannabidiol and cannabinol, as well as THC-COOH as metabolite of THC. THC-COOH concentrations in hair samples are very low, so analysis of THC alone is more frequent in laboratory practice.

OBJECTIVES: Our goal was to develop fast and reliable LC-MS method for determination of THC in hair samples.

METHOD / DESIGN: Decontamination of hair samples was achieved by washing of hair samples in methylene chloride. After decontamination, hair sample was pulverized and tribenzylamine (TBA) as internal standard was added into the sample. Afterwards sample was solubilized in 1 M sodium hydroxide, and then incubated in water bath at 95°C for 10 minutes. THC was isolated from samples with hexane:ethylacetate mixture (9:1, v/v). Detection of THC was performed by LC-MS method in single ion monitoring mode (SIM) at m/z 315, 232 and 214 for THC, and 288 for TBA. The chromatographic separation was performed on XTerra®RP18 column, using a gradient of acetonitrile/formic acid 1% and formic buffer pH 3.5 as the mobile phase. Linearity was achieved in the range from 0.5-4 ng/mg.

RESULTS: Retention times of THC and TBA were 16.78 and 21.84 minutes, respectively. Limit of detection and limit of quantitation for THC were 0.0076 and 0.025 ng/mg respectively. Coefficient of variation was 0.59%.

CONCLUSIONS: Hair analysis found appliance in forensic toxicology because of possibility to analyze retrospective exposure to drug or some other substance, when blood and urine are no longer expected to contain that particular chemical. Also it is very useful for workplace drug testing and for police investigation purposes. Due to these reasons, and considering rise in Cannabis consumption, we developed fast and economical method that can answer to all challenges of drug testing.

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CANCER CELL GROWTH INHIBITING AND PROAPOPTOTIC PROPERTIES OF NATURAL FURANOACRIDONES

Istvan Zupko¹, Zsuzsanna Schelz¹, Imre Ocsovszki², Noémi Bózsity¹, Judit Hohmann³

¹ Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

² Department of Biochemistry, University of Szeged, Szeged, Hungary

³ Institute of Pharmacognosy, University of Szeged, Szeged, Hungary

*Corresponding author: zupko@pharm.u-szeged.hu

KEYWORDS: acridone alkaloids, furanoacridones, antitumor effect, apoptosis

INTRODUCTION: Natural products including alkaloids play a crucial role in drug development which is especially true for anticancer agents. Acridone alkaloids are typical secondary metabolites of the Rutaceae family and are reported to exert anticancer effects by intercalating into DNA of the cancer cell.

OBJECTIVES: The aim of the present study was the evaluation of the antiproliferative property of a set of pharmacologically uncharacterized furanoacridone alkaloids isolated from *Ruta graveolens*. The most promising member of the set was selected for additional experiments in order to collect information on the mechanism of the action.

METHOD: The antiproliferative actions of the alkaloids were determined by means of MTT assay against a panel of human breast cancer cell lines (MCF-7, MDA-MB-361, MDA-MB-231 and T47D). Cancer selectivity was determined using non-cancerous immortalized cells and fibroblasts. Cell cycle analysis was performed by flow cytometry after propidium iodide (PI) staining. Morphological consequences of the treatments were documented by fluorescent microscopy after Hoechst 33258 – PI staining. The induction of apoptosis was confirmed by the colorimetric determination of the crucial caspases (caspase-3, -8 and -9).

RESULTS: Isogravacridone chlorine (IGC) proved to be the most potent of the tested alkaloids eliciting comparable or lower calculated IC₅₀ values than reference agent cisplatin. IGC exerted only a limited action on the proliferation of non-malignant cells. Treatment of triple-negative cancer cell line MDA-MB-231 with IGC resulted in a cell cycle disturbance and the accumulation of hypodiploid (subG1) population in a concentration- and time-dependent way. Nuclear condensation was additionally evidenced without substantial deterioration of cell membrane function. Finally, IGC increased the activities of caspase-3 and -9 in the treated MDA-MB-231 cells without influencing caspase-8 indicating the initiation of apoptosis via mitochondrial pathway.

CONCLUSIONS: These in vitro findings indicate that furanoacridones are suitable candidates for anticancer drug development.

Financial support from the Hungarian Scientific Research Fund (OTKA K-109293, I. Zupko) is gratefully acknowledged.

**INVESTIGATION OF ANTIPROLIFERATIVE PROPERTIES OF
13 α -ESTRONE DERIVATIVES IN VITRO**

Izabella Sinka¹, Ida Kovács¹, Erzsébet Mernyák², Imre Ocsovszki³,
Gyula Schneider², János Wölfling², István Zupkó¹

¹ Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

² Department of Organic Chemistry, University of Szeged, Szeged, Hungary

³ Department of Biochemistry, University of Szeged, Szeged, Hungary

*Corresponding author: sinkaiza@gmail.com

KEYWORDS: Antiproliferative effect; Apoptosis; 13 α -estradiol; Cell cycle; Caspases

INTRODUCTION: The development of anticancer drugs based on naturally occurring biologically active substances is one of the most relevant approach of current drug development. Although 17 β -estradiol has been described as a proliferation-inducing compound, recent experimental results indicated that certain of its analogs can exert potent antiproliferative effects without appreciable estrogenic activity.

OBJECTIVES: The aim of the current investigation was to characterize the antiproliferative properties of newly synthesized set of 13 α -estrone derivatives on various human reproductive cancer cell lines *in vitro*. These compounds do not exhibit appreciable estrogen receptor binding affinity and therefore no estrogenic activity, because of their 13 α -methyl substituent.

METHOD: The growth-inhibitory effects of these compounds were determined on four malignant cell lines (HeLa, A2780, A431, MCF-7) by means of MTT-assay. To investigate the mechanism of the antiproliferative activity of the most promising compound cell cycle analysis by flow cytometry has been performed. Morphological changes were detected by Hoechst 33258 – propidium iodide double staining which allowed the identification of intact, early-apoptotic, late-apoptotic and necrotic cells. To specify the mechanism of action caspase-activity was measured by colorimetric method with specific substrates.

RESULTS: 2 of the 18 examined substances were exerted potent antiproliferative activity, which was comparable with the clinically used anticancer agent cisplatin. The number of cells in the G2/M phase was significantly higher after treatment for 24 hours on the expense of G1 cell population. After 48 hours of treatment a significant increase was detected in the number of hypodiploid (subG1) cells. Furthermore, apoptosis inducing effect was clearly evidenced by morphological examination and the significant increases in the activity of caspase-3 and caspase-9. These results indicate a blockade of the cell cycle at the G2/M transition and apoptosis induction via mitochondrial pathway.

CONCLUSIONS: On the basis of the presented results D-ring modified estrone derivatives can be regarded as a new group of apoptosis-inducing antiproliferative agent. Financial support from the Hungarian Scientific Research Fund (OTKA K-109293, I. Zupko) is gratefully acknowledged.

COMPOSITION AND ANTIOXIDANT ACTIVITY OF “SAMONIKLICA” APPLE JUICE AND JAM

Mirjana Amidžić¹, **Jasna Jablan**¹, Barbara Fumić¹, Adela Krivohlavek², Senka Ninković², Marijana Zovko Končić¹

¹ Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

² Nastavni zavod za javno zdravstvo-dr.Andrija Štampar, Mirogojska 16, 10000 Zagreb, Croatia

*Corresponding author: Jasna Jablan: jjablan@pharma.hr

KEYWORDS: “samoniklica” apple; iron; antioxidant; polyphenol.

INTRODUCTION: Jam of indigenous varieties of “samoniklica” apple is traditionally used in Bosnia and Herzegovina for strengthening the body, improving immunity and as a natural remedy for the treatment of iron deficiency, especially in pregnancy. Although juice and jam are potentially rich in iron and phenolic antioxidants, a high amount of sugar in them may be associated with oxidative stress. Furthermore, apples could be contaminated with heavy metals from the environment.

OBJECTIVES: The aim of this study was to examine the justifiability of juice and apple jam use for traditional tonic purposes, as well as in the prevention and treatment of iron deficiency anemia, and assess the benefits and risks of such an application.

METHOD / DESIGN: For this purpose, the composition of phenolic antioxidants (total phenol, total flavonoids and phenolic acids), as well as total sugar content, were determined spectrophotometrically. Antiradical and iron chelating activity of the samples were determined by using DPPH free radical and ferrozine, respectively. The atomic absorption spectroscopy was used to assess the quantity of iron and other heavy metals.

RESULTS: The tested samples contained relatively small amounts of phenolic antioxidants. Jam contained more total polyphenols (6.44 mg/g vs. 1.92 mg/g) and phenolic acids (1.18 mg/g vs. 0.06 mg/g) than juice. The amount of total flavonoids (41.09 µg/g vs. 19.47 µg/g) and sugars (79.2 mg/g vs. 58.5 mg/g) was, on the other hand, higher in the juice than in the jam. Both juice (0.69 mg/ml) and jam (5.76 mg/ml) were weaker radical scavengers than BHA (0.01 mg/ml) or ascorbic acid (0.02 mg/ml). Similarly, the chelating activity of the juice (8.49 mg/ml) and jam 0.46 (mg/ml) was lower than the activity of EDTA (2.0 µg/g) or quercetin (9.6 µg/g). Both samples contained iron, which was more abundant in juice. However, the amount of iron in 15 ml of jam, which is traditionally taken for iron deficiency, contains much lower dose of that mineral than the recommended daily dose of 18 mg/day. Furthermore it was found that the lead content deviates from the value prescribed by law, which can have negative effects on the organism, especially during pregnancy and lactation.

CONCLUSIONS: The results demonstrate that the use of “samoniklica” jam in the prevention and treatment of iron deficiency anemia is not justified.

CHEMICAL COMPOSITION AND α -GLUCOSIDASE INHIBITORY ACTIVITY OF PLANTS TRADITIONALLY USED FOR MANAGEMENT OF DIABETES IN CROATIA

Kristina Bljajić¹, Lovorka Vujić¹, **Jasna Jablan**¹, Marijana Zovko Končić¹

¹ Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

*Corresponding author: Jasna Jablan: jjablan@pharma.hr

KEYWORDS: Diabetes; α -glucosidase inhibitory activity; heavy metals; polyphenols.

INTRODUCTION: Type 2 diabetes affects 90% of diabetics and is largely the result of excess body weight and physical inactivity. The treatment consists of lowering blood glucose levels and preventing the development of secondary diabetic complications.

OBJECTIVES: Treatment of type 2 diabetes by traditional medicine is common in Croatia. The aim of this study was to investigate potential usefulness of several plants traditionally used for management of diabetes by examining their chemical composition and α -glucosidase inhibitory activity. In order to eliminate the potential of influence of heavy metals on the enzyme activity, their amounts in samples were also determined.

METHOD / DESIGN: For this study, 7 medicinal plants traditionally used in treatment of diabetes were selected: *Artemisia absinthium* (leaf), *Achillea millefolium* (aerial parts), *Betula pendula* (leaf), *Centaurium erythraea* (aerial parts), *Morus sp.* (leaves), *Salvia officinalis* (leaf) and *Sambucus nigra* (flower). Aqueous and ethanolic extracts of selected plants were prepared. Phenolic composition of the extracts was determined using RP-HPLC with UV and/or DAD detection, while α -glucosidase inhibitory was determined spectrophotometrically. Heavy metals concentration was determined using the bench top Total reflection X-ray Fluorescence (TXRF) spectrometer "S2 Picofox" (Bruker Nano GmbH, Berlin, Germany).

RESULTS: HPLC analysis shown that the selected medicinal plants contain a variety of phenolic acids while the investigated flavonoid derivatives were less common. Most of investigated samples (6 from 7) contained caffeic acid and/or its derivative, chlorogenic acid. Knowing that these cinnamic acid derivatives may, even in low concentrations, prevent glucose-induced endothelial dysfunction associated with inflammation and oxidative stress, it may be concluded that the plants that contain them may contribute to the prevention of secondary complications of diabetes. Furthermore, all the prepared extracts exhibited α -glucosidase inhibitory activity. Among the investigated samples *Morus sp* and *B. pendula* extracts were the most active. In this investigation TXRF analysis was successfully applied for the multielemental analysis. The levels of investigated metals in all analyzed samples were in the permitted concentrations.

CONCLUSIONS: Plants used for management of diabetes in Croatia possess α -glucosidase inhibitory activity. Furthermore, they contain numerous bioactive polyphenols, caffeic acids and its derivatives being the most prominent examples. The content of heavy metals in all prepared extracts were bellow maximal permissible concentrations. The performed research indicates that the investigated medicinal plants may possess significant potential in treatment of diabetes and its complications.

ROSA SEMPERVIRENS L. ROSE HIPS AS A PROMISING SOURCE OF ANTIOXIDANT AGENTS

Zorica Mrkonjić¹, Jelena Nađpal¹, Filip Šibul¹, Tatjana Majkić¹,
Diandra Pintać¹, Marija Lesjak¹, Ivana Beara¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

*Corresponding author: zoricamrkonjic@gmail.com

KEYWORDS: *Rosa sempervirens* L.; antioxidant activity; vitamin C; phenolics;

INTRODUCTION: *Rosa sempervirens* L. is native plant of the Mediterranean region, where its rose hips have been traditionally used in treatment of malaria, hemorrhoids, hepatitis, stomach ache, bronchitis and diarrhea [1]. In addition, due to great similarity to other more recognized species from *Rosa* genus (e.g. *Rosa canina*), it is often unknowingly used for different food preparations. Regardless of widespread traditional usage of *Rosa* species in cooking and medicine, only *R. canina* was examined in detail, while there were only a few reports about bioactivity and chemical composition of other *Rosa* species, such as *R. sempervirens*.

OBJECTIVES: The aim of this study was to examine antioxidant potential, vitamin C, total phenolic and flavonoid contents, as well as phenolic profile of water and methanol extracts of air-dried rose hips of *R. sempervirens*.

METHOD / DESIGN: Rose hips were collected in May 2014, in Čanj, Montenegro. Water and methanol extracts of air-dried rose hips were prepared by maceration. Antioxidant activity of extracts and well-known synthetic antioxidant propyl gallate (PG) was evaluated using several *in vitro* assays by measuring radical scavenging capacity of diphenylpicrylhydrazyl (DPPH[•]), superoxide anion (O₂^{•-}) and hydroxyl (HO[•]) radicals, nitric oxide (•NO), as well as reducing power (FRAP) assay and inhibition of Fe²⁺/ascorbate induced lipid peroxidation. In addition, vitamin C, total phenolic and flavonoid contents were determined spectrophotometrically. Determination of 44 plant phenolics was performed using LC-MS/MS technique.

RESULTS: All determined antioxidant activities of extracts and PG were concentration dependent. Overall antioxidant activity of extracts was considerable and comparable with PG activity. Water extract showed better ability in inhibition of lipid peroxidation and exhibited better reduction power than methanol extract, while methanol extract was more effective in scavenging DPPH[•], O₂^{•-} and HO[•] in comparison with water extract. However, both extracts were inactive in scavenging •NO. Furthermore, in both extracts significant content of vitamin C was determined (2.12 mg/g of dry weight (dw) in water extract and 1.58 mg/g of dw in methanol extract). Also, notable content of total phenolics were found in water extract (84 mg of gallic acid equivalents (GAE)/g of dw, while in methanol extract this content was significantly lower

(58mg of GAE/g of dw). Total flavonoid content was low in both extracts. LC-MS/MS analysis identified presence of 12 from 44 investigated phenolics. Gallic acid was the most abundant phenolic acid, while among 25 investigated flavonoids, catechin content was the highest in both extracts, especially in methanol extract. Also, glycosides such as quercitrin, quercetin-3-O-glucoside, hyperoside, kaempferol-3-O-glucoside were present in notable amounts.

CONCLUSIONS: Obtained results showed that *R. sempervirens* hips present a valuable source of compounds with high antioxidant potential. Furthermore, the presented results strongly support the usage of *R. sempervirens* hips as a food with health benefits and traditional remedy.

Acknowledgement: The Ministry of Education and Science of Republic of Serbia (Grant No.172058) supported this research work.

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ROSA DUMETORUM THUILL HIPS AND FOOD PRODUCTS AS A SOURCE OF PHENOLIC COMPOUNDS AND ACETYLCHOLINESTERASE INHIBITORS

Jelena Nađpal¹, Zorica Mrkonjić¹, Mirjana Ćuk², Tatjana Majkić¹,
Diandra Pintać¹, Marija Lesjak¹, Ivana Beara¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg D. Obradovića 3, 21000 Novi Sad, Serbia

² University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg D. Obradovića 3, 21000 Novi Sad, Serbia

*Corresponding author: jelena.nagypal@outlook.com

KEYWORDS: *Rosa dumetorum* Thuill, phenolic profile, acetylcholinesterase activity

INTRODUCTION: Rose hips are highly recognized in ethnomedicine mostly due to their prophylactic and therapeutic properties against wide range of disorders, such as inflammation, arthritis, flu, vitamin C deficiency, diabetes, gastrointestinal, kidney and lower urinary tract disorders. Also, they have been widely consumed as tea, jelly, jam, soup and beverages. Even though the genus *Rosa* comprises about 200 species [1], chemical composition and biological activity of only a few *Rosa* species have been investigated. Rose hips of *Rosa dumetorum* Thuill have been used all over Europe as a traditional remedy and food. Only few previous studies of *R. dumetorum* rose hips indicate that they could be a valuable source of phenolics and other health promoting compounds, while their biological activity remained uninvestigated.

OBJECTIVES: The aim of present study was to examine in detail phenolic profile and acetylcholinesterase (AChE) inhibitory activity of purée, jam, water and methanol extracts of fresh and air-dried rose hips of so far poorly explored *R. dumetorum*.

METHOD / DESIGN: Rose hips of *R. dumetorum* were collected in November 2013, in Deliblatska peščara (Deliblato sands), Republic of Serbia. Water and methanol extracts of fresh and air-dried rose hips were prepared by maceration, while purée and jam were made according to traditional recipes. In detail examination of phenolic profile included quantitative analysis of 14 phenolic acids, 25 flavonoids, 3 coumarins and 2 lignans by highly sensitive and specific LC-MS/MS technique. AChE inhibitory activity was evaluated using *in vitro* assay based on modified Ellman's spectrophotometric method [2].

RESULTS: LC-MS/MS analysis of selected phenolics resulted in quantitative determination of 11 among 44 examined compounds. All extracts showed similar phenolic profile, but quantitative differences of phenolics between the extracts was evident. Among examined phenolic acids, gallic and protocatechuic acids were the most abundant. The most dominant flavonoid was catechin, while notable content of quercetin glycosides (quercitrin, quercetin-3-*O*-glucoside and hyperoside) were also confirmed. Examined coumarins and lignans were not present in investigated extracts. Methanol extract of fresh hips had the highest content of investigated phenolics (6.46 mg/g of

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dry weight (dw)), while the lowest content was detected in jam extract (1.45 mg/g of dw). In terms of AChE inhibitory activity, all extracts apart from jam, showed concentration dependent inhibitory activity. Overall, extracts exhibited moderate activity in comparison with galantamin, a well known AChE inhibitor from plants. Specifically, methanol extract of fresh hips expressed the highest AChE inhibitory potential and water extract of air-dried hips the lowest ($IC_{50} = 1.30$ and 7.97 mg/mL, respectively).

CONCLUSIONS: Presented results indicate that *R. dumetorum* rose hips possess moderate AChE inhibition potential and that they are valuable source of phenolics. This study justify usage of *R. dumetorum* rose hips in diet and ethnomedicine as a fruitful source of compounds with health benefits. In addition, further studies of their chemical composition and biological activities are supported.

Acknowledgement: The Ministry of Education and Science of Republic of Serbia (Grant No.172058) supported this research work.

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GARLIC MUSTARD (*ALLIARIA PETIOLATA*) – UNREWARDED SPICE WITH ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES

Jelena Katanić¹, Tatjana Boroja¹, Stefanie Nikles², San-Po Pan², Vladimir Mihailović¹, Rudolf Bauer²

¹ Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia

² Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria

*Corresponding author: jkaranic@kg.ac.rs (J. Katanić)

KEYWORDS: *Alliaria petiolata*; antioxidant activity; anti-inflammatory activity; phenolic content

INTRODUCTION: *Alliaria petiolata* (garlic mustard, Brassicaceae) is a biennial flowering plant native to Europe, but also invasive in many geographical areas. The main class of secondary metabolites produced by the Brassicaceae family is the glucosinolates and this plant contains the glucosinolate sinigrin and alliarinoside, a γ -hydroxynitrile glucoside structurally related to cyanogenic glucosides. Sinigrin may defend this plant against a broad range of enemies, while alliarinoside confers resistance to specialized (glucosinolate-adapted) herbivores. Moreover, all glucosinolate-containing plants from Brassicaceae family contain enzyme myrosinase which catalyze the hydrolysis of glucosinolates to isothiocyanates, responsible for the typical odor and taste of cruciferous vegetables. All parts of *A. petiolata*, including the roots, give off a strong odour, a mixture of mustard and garlic. *A. petiolata* flowers are in usage in traditional medicine for healing wounds, as expectorant, antiseptic, and stimulant.

OBJECTIVES: The aim of our research was to evaluate total phenolic composition (total phenolics, flavonoids, flavonols, and phenolic acids) of methanolic extracts of herba (APA) and roots (APR) of *A. petiolata*, as well as their antioxidant and anti-inflammatory potential.

METHOD / DESIGN: Antioxidant properties were determined by superoxide anion radical, DPPH radical and ABTS cation radical scavenging assays. Anti-inflammatory activity was evaluated via cyclooxygenase-1 and -2 inhibition assays and COX-2 gene expression determination.

RESULTS: Total phenolic content of APA extract (331.55 mg GA/g) was more than two fold higher than in APR (152.74 mg GA/g). Also, contents of flavonoids, flavonols, and phenolic acids were much higher in aerial part extract. In root extract (APR), flavonols and phenolic acids were detected in traces. Antioxidant potential of APA was much more pronounced in all applied assays, with IC₅₀ values in a range from 108.71 to 459.95 $\mu\text{g/mL}$. IC₅₀ values for APR were much higher, e.g. 727.11 $\mu\text{g/mL}$ for ABTS, and 755.88 $\mu\text{g/mL}$ O₂⁻ scavenging activity. Anti-inflammatory potential of these two extracts were more pronounced. APA and APR, in concentration of 50

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µg/mL were able to inhibit COX-1 enzyme for 67.48 and 46.15 %, respectively. More significant were the results for COX-2 enzyme inhibition, 76.94% for APA and 59.02% for APR. The results of COX-2 gene expression assay were negative and thus showed that inhibition of COX-2 gene expression was not the main route for APA and APR anti-inflammatory activity.

CONCLUSIONS: The obtained results showed that *A. petiolata* aerial part and root methanolic extracts contained significant amount of phenolic compounds. APA extract exerted much better antioxidant activity than APR. On the other hand, the anti-inflammatory potential of *A. petiolata* extracts was remarkable. Presented findings could lead to the isolation and identification of compounds in *A. petiolata* aerial parts or roots that are responsible for the anti-inflammatory properties of this plant and thus ensure its use in some pharmaceutical formulations.

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MULTI-RESPONSE OPTIMIZATION OF ULTRASOUND-ASSISTED GLYCEROL EXTRACTION OF BIOACTIVE BERBERINE FROM *BERBERIS VULGARIS*

Marina Dalić¹, **Marijana Zovko Končić**¹

¹ Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10000 Zagreb, Croatia

*Corresponding author: Marijana Zovko Končić mzovko@pharma.hr

KEYWORDS: *Berberis vulgaris*; berberine; radical scavenging activity; response surface methodology.

INTRODUCTION: Barberry (*Berberis vulgaris* L., Berberidaceae) is a well known medicinal plant with a long history of medicinal and nutritional use in Europe, Asia and America. It has various therapeutic effects, mostly related to its isoquinoline alkaloids, particularly berberine.

OBJECTIVES: The aim of this study was to examine the efficiency of ultrasound-assisted extraction of berberine from *Berberis vulgaris* using glycerol as non-toxic, biodegradable solvent from renewable sources.

METHOD / DESIGN: Experiment was planned using Box-Behnken design. The range of design parameters (independent variables) was: temperature (X_1 , 20-80°C), glycerol concentration (m/m) (X_2 , 10-90%) and ultrasonication power (X_3 , 72-720 W). Berberine content of the extracts was determined using RP-HPLC-DAD, while radical scavenging activity of the extracts (RSA) was determined spectrophotometrically, using DPPH free radical (dependent variables). Response-surface methodology was used to find the relationship between dependent and independent variables.

RESULTS: The relationship between variables can best be described by quadratic models. The selected quadratic models were highly significant ($P > 0.01$) and had r^2 values over 0.93. The most important extraction variables were temperature and glycerol content. They significantly ($P > 0.05$) influenced berberine concentration and RSA both as linear and quadratic terms. Furthermore, ultrasonication power had a significant ($P < 0.05$) effect on berberine concentration as a quadratic term, as well as in interaction with the extraction temperature. The solutions to the quadratic equations have shown that the best extraction conditions are (X_1 , X_2 , X_3) for berberine extractions are 80°C, 50% glycerol and 144 W, while the extracts showing best RSA were obtained using 80 °C, 40% glycerol and 720 W. The experimental data has confirmed these findings.

CONCLUSIONS: The results demonstrate that ultrasonication-assisted extraction using glycerol is a fast and efficient method for preparation of extracts with notable berberine concentration and radical scavenging activity.

ANTIBIOFILM ACTIVITY OF COPPER(II)-COMPLEXES WITH SOME S-ALKYL DERIVATIVES OF THIOSALICYLIC ACID

Marina Ž. Mijajlović¹, Andriana M. Bukonjić¹, Dušan Lj. Tomović¹, Aleksandar Kočović¹, Miloš V. Nikolić¹, Verica V. Jevtić², Zoran R. Ratković², Ivana D. Radojević³, Jovana Z. Maksimović³, Sava M. Vasić³, Ljiljana R. Čomić³, Srećko R. Trifunović², Gordana P. Radić¹

¹ Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Republic of Serbia,

² Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia,

³ Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia

*Corresponding author: vasic_gordana@yahoo.com (G.P. Radić)

KEYWORDS: copper (II)-complexes; thiosalicylic acid; antibiofilm activity; tissue culture plate method.

INTRODUCTION: The recognition of copper(II)-complexes as important bioactive compounds increased the interest in these agents as potential drugs for various diseases. Current investigations in copper bioinorganic chemistry are focused on potential use of copper coordination compounds as antimicrobial, antiviral, anti-inflammatory, antitumor agents and enzyme inhibitors.

OBJECTIVES: The aim of the study was *in vitro* antibiofilm screening of five copper(II)-complexes with some S-alkyl derivatives of thiosalicylic acid.

METHOD / DESIGN: The influence on bacterial biofilm formation was determined by tissue culture plate (TCP) method. We screened all tested species for their ability to form biofilm by TCP method with some modifications. Biofilm inhibitory concentration (BIC) was defined as the lowest concentration of each complex where the biofilms were dispersed. This method is with crystal violet and doxycycline as positive control. The inoculated plates were incubated at 37°C for 24 h for Gram-negative bacteria and 48 h for Gram-positive bacteria. The concentrations of the complexes were from 62.5 up 1000 µg/mL.

RESULTS: Biofilm inhibitory concentration (BIC) values were in the range from 125 to >1000 µg/mL, depending on the bacterial species and the test substance. Gram-positive bacterial biofilm showed a higher sensitivity than the Gram-negative. The most sensitive bacterial biofilm was *Staphylococcus aureus* with BIC value of 125 µg/mL and MIC value of 250 µg/mL.

CONCLUSIONS: Comparing the results with the results of the positive control (doxycycline) it can be concluded that the tested complexes generally showed lower activity, however all copper(II)-complexes had a higher antibiofilm activity than doxycycline on *Staphylococcus aureus*.

COMPARATIVE ANALYSIS OF THE CHEMICAL CHARACTERIZATION OF CRATAEGUS SPECIES FROM SERBIA AND ROMANIA

Marija Čanković¹, Snezana Cupara¹, Marina Tomović¹, Ana Radovanović¹, Viorica Tamaș², Marija Popović Milenković³, Slobodan Janković⁴

¹ Pharmacy Department, Faculty of Medical Sciences, University of Kragujevac, Serbia,

² S.C. Hofigal Export – Import S.A., Bucharest, Romania,

³ Community Pharmacy Kragujevac, Serbia.

⁴ Pharmacology and Toxicology Department, Faculty of Medical Sciences, University of Kragujevac, Serbia.

*Corresponding author: maracankovic@gmail.com

KEYWORDS: *Crataegus*; Flavonoids; Polyphenols; Procyanidins; Antioxidant.

INTRODUCTION: *Crategus* species (Rosaceae) have been investigated for beneficial effects on the level of cholesterol, triglycerides, low-density lipoprotein, and glycemia. Traditionally it has been used for its positive effects on coronary artery flow and as a mild anxiolytic. Leaves and fruits of *Crategus* species have similar chemical composition. They are rich in various bioflavonoid complexes which are considered to be mostly responsible for cardiotonic effect of these plants. We have investigated chemical compositions of *Crataegus pentagina*, *Crataegus monogina* and *Crataegus nigra*.

OBJECTIVES: The aim of this study was to compare the chemical composition of three different *Crategus* species (*C. monogyna*, *C. nigra* and *C. pentagyna*) collected from different locations in Serbia and Romania.

METHOD / DESIGN: Plant material consisted of flowers, buds, leaves and fruits. Most examined samples were collected in Romania except fruits of *C. nigra* and *C. monogynae* fruits which originated from Serbia. The aqueous extracts were prepared from dried material by standard decoction method. The following methods were used for determination of total content of flavonoids (expressed as percent of rutin). The content of flavonoids was determined the use of using a UV / VIS spectrophotometer; employing the standard curve method, and the absorbances of the herbal extract dilutions were measured at 430 nm. The polyphenol carboxylic acid content was expressed as the per cent of caffeic acid. The UV/VIS spectrophotometric method was used, and the absorbances of the extracts were measured at 660 nm. The content of procyanidins was calculated using the method described in European Pharmacopoeia 6.0 and expressed as equivalent of cyaniding chloride. The total carbohydrates were determined using the standard Anthrone method, which is based on the hydrolysis of carbohydrates into simple sugars by dilute hydrochloric acid. The dehydration of glucose produces hydroxymethyl furfural, which after reacting with the Antron reagent, could be measured at 630 nm. Carbohydrates were expressed as percent of glucose. Antioxidant activity was determined by the use CUPRAC method (expressed as g of Trolox/g).

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RESULTS: The highest value of total flavonoids - 3,73 % was observed in *C. pentagynae* leaf,- while the lowest value was find in the fruits of *C. nigra* (0,79 %). The buds of *C. pentagynae* and leaves of *C. monogynae* contained the highest amount of polyphenolic acids 2,70% and 3,32 % respectively, while the lowest one was in fruits of *C. nigra* (0,9 %). *C. monogyna* fruits from Serbia had the highest content of carbohydrates (30,62 %), while the lowest content of carbohydrates was found in *C. monogyna* fruits from Romania (18,10 %). The highest concentration of procyanidins (4,97%), was found in the fruits of *C. pentagyna*, while the lowest one was found in *C. monogyna* fruit from Serbia (1,60%). All extracts showed antioxidant activity, but the extract of *C. monogyna* fruit obtained from Romania had the highest antioxidant activity.

CONCLUSIONS: The content of examined classes of phenolic compounds and carbohydrates in all three *Crataegus* species was in accordance with literature data for this plant material.

INVESTIGATION OF THE ESSENTIAL OILS OF *SALVIA OFFICINALIS*, *S. FRUTICOSA*, *MELISSA OFFICINALIS*, AND THEIR INFUSIONS

Aikaterini Koutsaviti¹, **Maria Couladis**¹

¹ *Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, Panepistimiopouli Zographou 157 71, University of Athens, Athens, Greece*

*Corresponding author: kouladi@pharm.uoa.gr

KEYWORDS: *Salvia*; *Melissa*; GC-M; Essential oils; Infusions.

INTRODUCTION: Many herbs with potentially beneficial effects which are attributed to their volatile constituents are used as herbal teas. *Salvia officinalis* L. (Lamiaceae) leaf is a traditional herbal medicinal product for symptomatic treatment of mild dyspeptic, complaints such as heartburn and bloating. *Melissa officinalis* L. (Lamiaceae) leaf is a traditional herbal medicinal product for relief of mild symptoms of mental stress and to aid sleep.

OBJECTIVES: The aim of this study was to determine the chemical composition of *S. officinalis*, *S. fruticosa*, and *M. officinalis* essential oils, as well as their infusions' volatiles.

METHOD / DESIGN: The essential oils and their infusions' volatiles were analyzed by means of GC and GC/MS.

RESULTS: *S. officinalis*: The results revealed that the oxygenated monoterpenes 1,8-cineole (27.5%) and camphor (11.5%) were the most abundant. *S. fruticosa*: also contained mainly oxygenated monoterpenes with α -thujone (16.5), β -thujone (16.4) and 1,8-cineole (8.8%) being the main constituents. *M. officinalis*: was characterized by the presence of a significant sesquiterpene fraction with caryophyllene oxide (14.9%) being the dominant component and by the presence of the oxygenated monoterpenes geranial (12.2%), neral (11.2%) and citronellal (6.7%).

CONCLUSIONS: Between the oils and the infusions volatiles there were no substantial qualitative differences, but there were quantitative variation.

PHYTOCHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *ARMERIA CANESCENS* (HOST) BOISS.

Valerija Dunkić¹, Mirko Ruščić¹, **Nada Bezić¹**

¹ Faculty of Science, University of Split, Croatia, 21000 Split, R. Boškovića 33

*Corresponding author: bezić@pmfst.hr

KEYWORDS: *Armeria canescens*; essential oils; phytol; piperitone; *p*-vinyl guaiacol

INTRODUCTION: The genus *Armeria* (Plumbaginaceae) is represented by about 120 species, provided that five species were recorded in the Balkan peninsula: *A. alpina* (DC.) Willd., *A. canescens* (Host) Boiss., *A. maritima* (= *A. vulgaris* Willd.), *A. rumelica* Boiss. and *A. vandasii* Hayek (Buzurović et al., 2015). In the area of Croatia are widespread several species, including research species *Armeria canescens* (Host) Boiss.. This species is the mesothermal halophytes widespread in Central Asia and the Mediterranean. In Croatia growing within the grassland association (as. *Armeria canescentis* - *Festucetum* Trinajstić et Sugar), which builds along with plant species *Festuca rupicola* and *Festuca valesiaca* Schl. These association lawn grows on the surface of sinkholes and karst fields along the Dinara mountains.

OBJECTIVES: This phytochemical study refers in the *A. canescens* (in Croatian „babinja svila“), with a specific focus on essential oil compounds A search of the literature dealing with secondary metabolites and morphological aspect of genus *Armeria* resulted with only a few articles (Buzurović et al., 2015; Gourguillon et al., 2015; Scasellati et al., 2016)..

METHOD / DESIGN: Plant material of *Armeria canescens* was collected from Tjarica (near Split) Croatia in the summer (July) 2013. Aerial parts of plants were performed in a shady place at room temperature for 10 days.

Dried aerial parts of plant material (100 g) were subjected to hydrodistillation for 3 h in Clavenger type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and 1 μ L was used for GC/FID and GC/MS measurements.

GC/FID analyses were performed on Varian 3900 gas chromatograph equipped with a flame ionization detector (FID) and VF-5ms capillary. GC/MS analyses were carried out on a Varian Saturn 2000 system equipped with a VF-5ms with similar temperature programmed as in GC.

The individual peaks were identified by comparison of their retention indices of *n*-alkanes to those of authentic samples and literature (Adams, 2007), as well as by comparing their mass spectra with the Wiley 9.0 library (Wiley, New York) and NIST/02 mass spectral database. The percentage composition of the samples was computed from the GC peak areas using the normalization method.

RESULTS: Water distilled essential oils from aerial parts of investigated plant have been analysed by GC and GC/MS using VF-5ms capillary column. Total yield of

oil was 0.05%, based on dry weight of sample. Thirty-one compounds, representing 77.9% of the total oil, were identified. The essential oil of *A. canescens* was characterized by a high concentration of diterpene alcohol phytol (29.2%). Among oxygenated monoterpene piperitone (9.4%) was the major compound, followed by an phenolic compound *p*-vinyl guaiacol (7.9%).

CONCLUSIONS: Previous research in the species *Armeria canescens* have included morphological and functional aspects of salt glands (Scassellati et al., 2016.).The present study gives additional knowledge about phytochemical composition of the essential oil on the genus *Armeria*.

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OPTIMIZATION OF THE EXTRACTION CONDITIONS FOR ISOLATION OF PHENOLIC COMPOUNDS FROM *PRUNUS AVIUM* STEMS

Nataša Nastić¹, Antonio Segura-Carretero^{2,3}, Jesús Lozano-Sánchez^{2,3}, Isabel Borrás-Linares^{2,3}, Jaroslava Švarc-Gajić¹

¹ Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia,

² Department of Analytical Chemistry, Faculty of Science, University of Granada, Avda. Fuentenueva s/n, 18071 Granada, Spain,

³ Functional Food Research and Development Centre (CIDAF), Health Science Technological Park, Avda. del Conocimiento s/n, Bioregion building, 18016 Granada, Spain

*Corresponding author: nat.nastic@gmail.com

KEYWORDS: Sweet cherry stems; *Prunus avium*; ASE; Total phenolic content

INTRODUCTION: The production of increasing amount of waste from various sources is one of the problems seen worldwide. In recent years, valorization of organic waste by different approaches has gained huge interest. In many cases, agro-food waste is high in valuable chemicals showing bioactive properties. Sweet cherry (*Prunus avium* L.) stems attract the interest of scientific community due to their long use in alternative medicine as sedative and diuretic in form of infusions and decoctions. Due to high content of flavonoids and potassium, it has been reported that cherry stems contribute to suppression of inflammation and have positive effects on cardiovascular system and smooth muscles. In order to develop adequate procedures for bio waste valorization, it is necessary to optimize extraction procedure targeting specific chemical classes.

OBJECTIVES: The aim of the present study was to optimize conditions for accelerated solvent extraction (ASE) technique to extract phenolic compounds from sweet cherry stems. Different combinations of solvent mixtures and temperatures were tested to optimize the extraction process with respect to extraction yields and total phenolic contents.

METHOD / DESIGN: *Prunus avium* stems were provided by a sweet cherry producer (*La Picota del Jerte, Spain*). The stems were air-dried at ambient temperature and grounded to fine powder. All ASE extractions were carried out in a static mode (100 bar; 20 min) for all tested combinations of extraction solvents (ethanol:water from 0% to 100%) at temperatures ranging from 40 to 200°C to cover a wide range of dielectric constants and polarities. After extraction, the residual solvent was evaporated using SpeedVac Concentrator. Total polyphenols in obtained extracts were determined spectrophotometrically by Folin-Ciocalteu method.

RESULTS: In this study, the extraction conditions (extraction solvent and temperature) for recovering bioactive compounds from sweet cherry stems in accelerated solvent extraction process were investigated and optimized for maximal yields of total phenols.

Table 1. Extraction yields and phenolic contents in *Prunus avium* stem extracts obtained by ASE technique

Ethanol in the mixture ethanol:water (%)	Temperature (°C)	Pressure (bar)	Time (min)	Extraction yield (%)	TPC (mg GAE/mg sample)*
50	40	100	20	16.92	0.217 ± 0.004
15	63	100	20	18.95	0.106 ± 0.008
85	63	100	20	10.03	0.054 ± 0.006
100	120	100	20	16.21	0.098 ± 0.004
0	120	100	20	10.54	0.029 ± 0.010
50	120	100	20	28.09	0.255 ± 0.008
15	176	100	20	37.31	0.155 ± 0.014
85	176	100	20	16.53	0.077 ± 0.010
50	200	100	20	31.08	0.088 ± 0.006

*The values shown are the mean ± 2SD of three replications.

CONCLUSIONS: The potential of sweet cherry by-product as a natural source of phenolic compounds has been evaluated using accelerated solvent extraction. The best extraction protocol to extract phenolic fraction from sweet cherry stems included ethanol/water mixture (50:50, v/v) at 120°C. The results from this work demonstrate optimized ASE protocol for obtaining natural extracts from sweet cherry stems enriched in polyphenols.

EXTRACTION OF *MORUS NIGRA* LEAVES BY DIFFERENT EXTRACTION TECHNIQUES

Nataša Nastić¹, Antonio Segura-Carretero^{2,3}, Jesús Lozano-Sánchez^{2,3}, Isabel Borrás-Linares^{2,3}, Jaroslava Švarc-Gajić¹

¹ University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia,

² Department of Analytical Chemistry, Faculty of Science, University of Granada, Avda. Fuentenueva s/n, 18071 Granada, Spain,

³ Functional Food Research and Development Centre (CIDAF), Health Science Technological Park, Avda. del Conocimiento s/n, Bioregion building, 18016 Granada, Spain

*Corresponding author: nat.nastic@gmail.com

KEYWORDS: Black mulberry; *Morus nigra*; ASE; SFE; Total phenolic content

INTRODUCTION: Black mulberry (*Morus nigra* L.) is deciduous plant widely spread in tropical, subtropical and temperate zones and well known for its nutritional qualities and medicinal properties. The extracts from different parts of mulberry tree (fruits, leaves and bark of the root) exhibit beneficial health effects, such as antiviral, anti-atherogenic and hypotensive. In addition, black mulberry leaves were reported to exhibit good glycemic control, inhibition of lipid peroxidation and the activity of catalase, indicating antihyperglycemic and antioxidant properties. The increasing interest in biological properties of black mulberry leaves have resulted in increased attention for different extraction techniques and their optimization.

OBJECTIVES: In the present study, the efficiency of the extraction of phenolic compounds from *Morus nigra* leaves is presented. Namely, accelerated solvent extraction (ASE) using water and ethanol as solvents, and supercritical fluid extraction (SFE) using supercritical CO₂ modified with ethanol, were compared in terms of extraction yields and total phenolic content (TPC). The effects of different operating conditions of these extraction techniques were investigated and defined.

METHOD / DESIGN: The black mulberry samples consisted of dried powdered black mulberry leaves obtained from local store (Novi Sad, Serbia). The samples were extracted by SFE and ASE. All SFE extractions were carried out at 40 °C in a dynamic mode with different solvent combinations (CO₂ with 7% and 15% of ethanol) and pressures (150 bar and 300 bar). According to previous kinetic studies, extraction time was set to 2 h to ensure high extraction yield. The collected extracts were concentrated at 40 °C using a rotary evaporator and reconstituted in different volumes of ethanol. The ASE experiments were performed in a static mode (100 bar and 20 min) with different combinations of solvent composition (ethanol: water from 0% to 100%) and temperatures (from 40 °C to 200 °C). After extraction, the residual solvent was evaporated using SpeedVac Concentrator. The total phenolic content in obtained extracts were determined by Folin-Ciocalteu method.

RESULTS: The main objective of the present study was to investigate the effects of process parameters (temperature and co-solvent percentage in SFE; temperature and extraction solvent in ASE) on the extraction efficiency, as well as to compare applied extraction techniques regarding the extraction yields and total phenolic contents.

Table 1. Extraction yields and phenolic contents in *Morus nigra* leaves

Extraction technique	Solvent	Temperature (°C)	Pressure (bar)	Time (min)	Extraction yield (%)	TPC (mg GAE/mg sample)*
SFE	CO ₂ + 7% ethanol	40	150	120	4.11	0.032 ± 0.004
	CO ₂ + 15% ethanol	40	150	120	6.07	0.049 ± 0.004
	CO ₂ + 7% ethanol	40	300	120	4.44	0.031 ± 0.004
	CO ₂ + 15% ethanol	40	300	120	6.90	0.042 ± 0.002
ASE	ethanol	120	100	20	11.74	0.042 ± 0.012
	ethanol:water (50:50)	40	100	20	18.72	0.078 ± 0.004
	ethanol:water (50:50)	120	100	20	25.05	0.159 ± 0.016
	ethanol:water (50:50)	200	100	20	48.29	0.251 ± 0.008
	water	120	100	20	13.40	0.021 ± 0.010

*The values shown are the mean ± 2SD of three replications.

CONCLUSIONS: Current paper describes optimization of the operational parameters of two advanced extraction techniques for the isolation of phenolic compounds from black mulberry leaves. ASE using the mixture of ethanol and water in ratio 50:50 at high temperature (200 °C) and pressure of 100 bar, provided the highest total phenolic content (0.251 mg GAE/mg sample) and the highest extraction yield (48.29%). The experiment also showed that the extraction yields and total phenolic contents produced by SFE were lower than those obtained by ASE. No significant differences were found in respect to co-solvent percentage and temperatures in SFE.

COMPARATIVE ANALYSIS OF TOTAL PHENOLICS CONTENT AND BIOLOGICAL POTENTIAL OF STINGING NETTLE (*URTICA DIOICA* L.) AND ROMAN NETTLE (*URTICA PILULIFERA* L.) EXTRACTS

Marina Francišković¹, **Nataša Simin**¹, Goran Anačkov², Dejan Orčić¹, Emilija Svirčev¹, Filip Šibul¹, Neda Mimica-Dukić¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000, Novi Sad, Serbia,

² University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000, Novi Sad, Serbia

*Corresponding author: natasa.simin@dh.uns.ac.rs

KEYWORDS: stinging nettle; Roman nettle; total phenolics content; antioxidant; anti-acetylcholinesterase

INTRODUCTION: *Urtica dioica* L. (stinging nettle) have been used as a leaf vegetable and in a traditional medicine primarily in the treatment of rheumatoid arthritis and as a diuretic [1]. Eventhough belonging to the same genus and having a lot of similarities with the stinging nettle, biological potential of Roman nettle (*U. pilulifera*) have never been assessed before.

OBJECTIVES: The aim of this work was to compare the total phenolics content, antioxidant and anti-acetylcholinesterase (AChE) activities of a widely known and used stinging nettle and poorly investigated Roman nettle.

METHOD / DESIGN: The 80 % methanolics extracts were made from herb and root for both species. Total phenolic content was determined with Folin–Ciocalteu reagent, antioxidant capacity was evaluated by the hydroxyl radical (HO[•]) neutralisation assay and the inhibition of lipid peroxidation (LP), while anti-AChE activity was examined by modified Ellman's method [2].

RESULTS: The results obtained are given in the Table 1. In both herb and root extracts of stinging nettle the determined total phenolics content was considerably higher compared to Roman nettle extracts. Nevertheless, Roman nettle herb extract exhibited better capacity in neutralizing HO[•] and inhibiting lipid peroxidation, thus, showing higher antioxidant potential. Contrary to that, stinging nettle root extract was better in both antioxidant assays. The AChE inhibitors are one of the main therapeutics in the treatment of Alzheimer's disease. In this work, stinging nettle herb extract exhibited highest inhibition of AChE (50%), while Roman nettle herb extract exhibited lower inhibitory potential (36%). Both nettle's root extracts were poor AChE inhibitors.

Table 1. The results of total phenolics content, antioxidant and anti-AChE activities of stinging and Roman nettle.

Extracts		Total phenolics mg eqGA/1g SE	HO [•] IC ₅₀ [µg/mL]	LP	anti-AChE I [%]
<i>Urtica dioica</i> L. (stinging nettle)	herb	104.0 ± 6.0	225 ± 12	211 ± 15	49.8 ± 1.2
	root	33.0 ± 3.0	532 ± 37	306 ± 10	16.5 ± 0.8
<i>Urtica pilulifera</i> L. (Roman nettle)	herb	74.4 ± 9.9	206 ± 24	190 ± 13	35.9 ± 1.3
	root	18.3 ± 3.0	620 ± 89	497 ± 35	17.4 ± 3.5

CONCLUSIONS: From the obtained results it can be concluded that the widely used stinging nettle has a higher phenolics content and capacity to inhibit AChE, but Roman nettle herb has a better antioxidant potential. With these results, the long and wide usage of stinging nettle as a food with health-promoting properties is confirmed. Additionally, a Roman nettle, whose biological activity was investigated for the first time, is proven to be a novel source of dietary antioxidants, therefore, should be implemented in a human consumption.

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THE EFFECT OF POLLUTANTS ON CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *TARAXACUM OFFICINALE* L.

Nataša Simin¹, Tamara Šipka¹, Emilija Svirčev¹, Milan Borišev²,
Marina Francišković¹, Kristina Bekvalac¹, Neda Mimica-Dukić¹

¹ University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000, Novi Sad, Serbia,

² University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000, Novi Sad, Serbia

*Corresponding author: 23tamara43@gmail.com

KEYWORDS: *Taraxacum officinale* L.; phenolic profile; antioxidant activity; pollution; heavy metal content.

INTRODUCTION: In this paper we investigated the influence of heavy metal content from the soil on the phenolic profile and antioxidant activity of the *Taraxacum officinale* L.

OBJECTIVES: The objectives of this paper were: to determine the degree of soil and *T. officinale* contamination by quantification of 15 heavy metals in both samples and to find potential correlation between them; to determine total phenolic and flavonoid contents of prepared plant methanol extracts using spectrophotometric method, and to quantify phenolics using LC-MS-MS analysis; to determine the antioxidant activity of plant extracts using several spectroscopic assays (scavenging abilities toward 'NO, HO' and DPPH', FRAP and inhibition of lipid peroxidation assays); to analyze potential correlation between degree of soil contamination, phenolic profile and antioxidant activity of plant extracts.

METHOD / DESIGN: Plant material was collected from two contaminated regions (refineries in Pančevo and Novi Sad, Serbia), and one uncontaminated region (Kačka forest in Kać, Serbia) and methanol extracts were prepared. Determination of heavy metals content in soil and plant material was done using ICP-MS technique where samples were prepared by microwave digestion with H₂O₂ and HNO₃ (system *Ethos, Microwave Labstation, Milestone*). The presence of 45 phenolic compounds in methanol plant extracts was investigated by LC-MS-MS analysis and the total phenolic and flavonoid contents were determined spectrophotometrically. Antioxidant activity was evaluated by standard spectrophotometric assays.

RESULTS: The analysis of heavy metal content showed that *T. officinale* L. does not accumulate heavy metals in its above-ground parts, but the content of Cu, Co and Fe was slightly increased in plant with the increase in content of those metals in corresponding soil. Using LC-MS-MS technique, 27 of 45 phenolic compounds were quantified. The concentration of luteolin and luteolin-7-O-glycoside was slightly lower in contaminated samples. Also, extracts from contaminated regions had lower total

phenolic and flavonoid contents, and lower antioxidant activity measured by all five assays.

CONCLUSIONS: *T. officinale* L. is not significant accumulator of heavy metals in its above-ground parts. The antioxidant activity and concentration of phenolic and flavonoid compounds in *T. officinale* L. decrease with soil contamination. This should be taken into account before cultivation of this plant. Also, regarding to these results, it can be concluded that *T. officinale* L. uses different strategies for protection from soil contamination, which do not include increase in concentration of phenolic secondary metabolites.

THE EFFECT OF APPLIED CROPPING SYSTEM (ORGANIC AND COVENTIONAL) ON ANTIOXIDANT PROPERTY OF FRESH JUICE OF SAVOY, RED AND WHITE CABBAGE

Svetlana Boskovic¹, **Neda Mimica-Dukić¹**, Dejan Orčić¹ and Nataša Simin¹

¹ University of Novi Sad, Faculty of Sciences, Department for Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

*Corresponding author: neda.mimica-dukcic@dh.uns.ac.rs

KEYWORDS: Brassica, antioxidant, flavonoids, free radicals, ABTS, CUPRAC

INTRODUCTION: The Brassica family comprises 350 genera and more than 3500 species. These plants are widely used as food, spices and sources of oils. Brassica vegetables are seen as highly protective against many chronic diseases and deserve special attention and presence in daily meals. Several secondary metabolites such as flavonoids, phenolic acids and glucosinolates are widely distributed in these plants species. Many of them were recognized to play chemo-preventive role and to be effective against free radicals damage and LDL oxidation, involved in pathogenesis of cardiovascular diseases, DNA damage and cancer.

OBJECTIVES: Numerous internal and external factors have an impact on the nutrient quality and health beneficiary of plants. Now days plants produced in organic cultivation are thought to be healthier, with higher content of health-promoting compounds. Thus, this study was undertaken to examine the effect of cropping conditions on the content of polyphenol compounds and antioxidant activities in fresh juices of three different cabbages.

METHOD / DESIGN: Savoy cabbage (*B.olearacea* L., var. *sabauda*), red cabbage (*B. oleracea* L. var. *rubra*) and white cabbage (*B.oleracea* L. var. *capitata*) were obtained from certified organic and conventional producers. From each plants fresh juice were prepared.

Total phenolic content (TPhC) was evaluated by the Folin-Ciocalteu colorimetric method and expressed in equivalents of gallic acid (mg GAE/L). Total flavonoid content (TFC) was determined spectrophotometrically by the aluminium chloride chelation metod and expressed in equivalents.of quercetine (mg QE/L). Antioxidant capacity was evaluated by measuring radical scavenging activity towards DPPH[•] and ABTS^{•+} radicals. Reduction potential was determined by CUPRAC assay. All results were expressed in mmol/L of TROLOX equivalents.

RESULTS: TPhC of red and white cabbages and TFC of Savoy and white cabbages increased under the influence of organic cropping conditions. Highest TPhC was found in red cabbage (2080 mg GAE/L), and the lowest in Savoy cabbage (1153 mg GAE/L). The highest TFC was found in conventionally grown red cabbage (183 mg QE/L), and the lowest in conventionally grown white cabbage (37.8 mg QE/L). TPhC and TFC

significantly differed among investigated subspecies ($p < 0.05$) while cropping conditions significantly affected only TFC of Savoy cabbage ($p < 0.01$). Scavenging capacity against DPPH and ABTS radicals and reduction potential measured by CUPRAC assay were significantly higher in red cabbage (10.4, 17 and 13.8 mmol TE/L; $p < 0.05$, respectively) than in other vegetables. Conventional cropping conditions improved antioxidant capacity of investigated vegetables except scavenging activity and reducing potential of red cabbage in ABTS and CUPRAC assays. However, influence of cropping conditions on scavenging activity of red cabbage against DPPH radicals ($p < 0.05$) as well as reducing potential of red and Savoy cabbages in CUPRAC assay ($p < 0.01$) was significant. The lowest scavenging activity was shown by organically grown Savoy cabbage- 0.6 mmol TE/L (in DPPH) and white cabbage- 3.1 mmol TE/L (ABTS) and 1.7 mmol TE/L (CUPRAC assay).

CONCLUSIONS: Results obtained show that in almost all examined plants TPhC and TFC were increased by organic while antioxidant capacity were mainly increased by conventional cropping conditions. Red cabbage can be distinguished as a plant with high amount of phenolic and flavonoid compounds and notable antioxidant capacity.

NEW ANTIPROLIFERATIVE ESTRONE DIMER DERIVATIVE

Noémi Bózsity¹, Viktória Nagy¹, Erzsébet Mernyák², Johanna Szabó²,
János Wölfling², István Zupkó¹

¹ Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6, H-6720.

² Department of Organic Chemistry, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary.

*Corresponding author: Noémi Bózsity (bozsity.noemi@pharm.u-szeged.hu)

KEYWORDS: estrogens, antiproliferative, proapoptotic, tubulin polymerization

INTRODUCTION: Estrogens are the most widely known and discussed hormones at women health science. Beside their role in reproduction, recently estrogen derivatives are widely investigated in cancer therapy. Even the natural 17 β -estradiol has been established as a tumor growth promoting hormone in numerous type of cancer, many new research revealed that some endogenous steroid metabolites inside the human body (2-ME, conjugated estrogens) have potent antiproliferative effect. This growth inhibiting metabolites inspired synthetic chemistry to design novel, more effective drug candidates with targeted modifications of the natural estrogens. Our previous results show that D-ring modified estrogens have pronounced anticancer effect in different reproductive cell lines.

OBJECTIVES: The aim of the present study was to investigate the antiproliferative activity of a novel dimer compound (DIM) synthesized by the linkage of a D-secoestrone and D-epiestrone compounds on three cervical cancer cell lines with different pathological backgrounds.

METHOD / DESIGN: The growth-inhibitory effects of DIM were determined by a standard MTT assay on HPV 18-positive HeLa, HPV 16-positive C33-A and HPV-negative SiHA cell lines. Fluorescent double staining have been performed to distinguish apoptotic and necrotic cells by their nuclear morphology and membrane integrity. The cell cycle phase distribution of the treated cells was investigated using flow cytometric analysis. The cell-independent direct effect of the test compound on tubulin polymerization was tested in vitro.

RESULTS: We have found that our test compound exerts a pronounced growth inhibitory effect on all of the tested cell lines, independently from their HPV status. After 24 hours incubation the cells showed the morphological signs of apoptosis visualized by fluorescent double staining. After incubation for 24 h, the DIM has no significant influence on cell cycle, but increasing the incubation time into 48 hours resulted significant changes of the cell cycle distribution with a similar trend on all of the cell lines. Regardless of the HPV status elevation of the apoptotic subG1 population was observed and significant increase was detected in the number of cells in G2/M, followed by a G1 phase reduction. Additionally, DIM significantly increased the maximum rate of microtubule formation, in vitro.

CONCLUSIONS: These results suggest that our new estrogen dimer derivative is a potent antiproliferative agent against cervical carcinoma cell lines. Regardless of the HPV status DIM exert proapoptotic effect proceeded by G2/M arrest and microtubule stabilization. These in vitro results confirm that the estrane skeleton is a potent model for designing new anticancer agents.

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SYNTHESIS AND 17 β -HSD1 INHIBITION OF HALOGENATED 13 α -ESTRONE DERIVATIVES

Ildikó Bacsa¹, Kevin Stefán Herman¹, Rebeka Jójárt¹, János Wölfling¹, Gyula Schneider¹, Bianka Edina Herman², Mihály Szécsi², Erzsébet Mernyák¹

¹ Department of Organic Chemistry, University of Szeged, Dóm tér 8, H6720 Szeged, Hungary

² Department of Medicine, University of Szeged, Korányi fasor 8–10, H6720 Szeged, Hungary

*Corresponding author: bacsa.ildike@gmail.com

KEYWORDS: 13 α -estrone; electrophilic aromatic halogenation; 17 β -HSD1 inhibition.

INTRODUCTION: Certain 2- and/or 16-haloestrones effectively inhibit the biotransformation of estrone into 17 β -estradiol, which is catalyzed by 17 β -hydroxysteroid dehydrogenase 1 (17 β -HSD1). Despite numerous efforts to design potent 17 β -HSD1 inhibitors, none have yet reached the stage of clinical trials because of their retained estrogenic activity. The availability of a 17 β -HSD1 inhibitor without hormonal behavior would be of particular interest. We published recently that certain A-ring halogenated 13 α -estrone derivatives display substantial 17 β -HSD1 inhibitory properties with IC₅₀ values in the submicromolar range. 13 α -Estrone is an appropriate scaffold for the design of biologically active estrone derivatives lacking hormonal behavior.

OBJECTIVES: Here we aimed to synthesize A- and/or D-ring halogenated derivatives in the 13 α -estrone series as potential 17 β -HSD1 inhibitors. 17-Deoxy-, $\Delta^{16,17}$ -13 α -estrone and their 3-ethers were chosen as starting compounds.

METHOD / DESIGN: Electrophilic halogenations were optimized using N-bromo- or N-iodosuccinimide in different solvents. The structures of the new compounds were confirmed by ¹H and ¹³C NMR measurements. The potential inhibitory action of the novel 13 α -estrones on human 17 β -hydroxysteroid dehydrogenase 1 activity was investigated via *in vitro* radiosubstrate incubation.

RESULTS: Several A- and/or D-ring halogenated derivatives of 13 α -estrone were effectively synthesized. Some compounds proved to be 17 β -HSD1 inhibitors, with IC₅₀ values in the submicromolar range.

CONCLUSIONS: Certain newly synthesized halogenated 13 α -estrones are promising 17 β -HSD1 inhibitors. Depending on the position of the introduced halogens, the compounds may possess dual inhibitory action against aromatase or steroid sulfatase enzyme.

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EVALUATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THYME (*THYMUS VULGARIS* L.) SUPERCRITICAL EXTRACTS

Sonja Roganović¹, Jelena Živković¹, Milica Stanković¹, Dragana Stojiljković², Svetolik Maksimović³, Vanja Tadić⁴, Ivana Arsić¹

¹ Department of Pharmacy, Faculty of Medicine, University of Niš, 81 Dr Zoran Đinđić Blvd., 18000 Niš, Serbia,

² Health Care Institution Pharmacy "Farmakop", 92 Nemanjić Blvd., 18000 Niš, Serbia,

³ Faculty of Technology and Metallurgy, University of Belgrade, 4 Karnegijeva St., 11000 Belgrade, Serbia,

⁴ Department for Pharmaceutical Research and Development, Institute for Medicinal Plant Research "Dr Josif Pančić", 1 Tadeuša Koščuška St., 11000 Belgrade, Serbia

*Corresponding author: sonja.naumovic@gmail.com

KEYWORDS: antioxidant activity; total phenolic content; supercritical extracts; *Thymus vulgaris*

INTRODUCTION: Extraction of plant material using supercritical fluids has been gaining popularity since the beginning of 20th century as a favorable technique for producing solvent-free extracts suitable for wide use in pharmaceutical, biomedical, cosmetic and food industries. Thyme (*Thymus vulgaris* L.) a significant aromatic plant is widely used for medicinal purposes as well as in culinary dishes.

OBJECTIVES: The aim of this study was to examine the antioxidant activity and total phenolic content of *Thymus vulgaris* supercritical extracts obtained using different extracting process parameters.

METHOD / DESIGN: Antioxidant activity of the extracts was measured on the basis of scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and Ferric Reducing Antioxidant Potential (FRAP) Assay. The content of total phenolics in extracts was determined by a modified Folin-Ciocalteu method.

RESULTS: The antioxidant activity and total phenolics of examined extracts are shown in Table 1.

Table 1. Total phenolics and antioxidant activity of *Thymus vulgaris* supercritical extracts

Sample	TPC ^A (mg GAE/g dried extract)*	DPPH ^B (IC ₅₀) (mg/mL)	FRAP ^C (mmol Fe ²⁺ /g dried extract)
<i>Thymus vulgaris</i> , 10 MPa/40°C	85.46 ± 0.41	> 1	0.36 ± 0.03
<i>Thymus vulgaris</i> , 30 MPa/40°C	117.77 ± 0.72	> 1	0.37 ± 0.02
<i>Thymus vulgaris</i> , 30 MPa/100°C	82.69 ± 0.25	> 1	0.49 ± 0.03
Ascorbic acid		0.0055 ± 0.014	

^A Total phenolics content (TPC) by Folin–Ciocalteu method, expressed in gallic acid equivalents (GAE).

^B DPPH radical scavenging activity.

^C Ferric reducing ability (FRAP).

* The experimental results were expressed as mean \pm standard deviation (SD) of three replicates.

CONCLUSIONS: On the basis of the obtained results, thyme has a potential use as natural antioxidant due to its significant antioxidant activity. The highest phenolic content and the strongest antioxidant activity exhibited the extract obtained using 30 MPa/40 °C during extraction process.

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ESSENTIAL OILS EFFECT ON THE *ACINETOBACTER BAUMANNII* PROTEIN LEAKAGE FROM CELLS

Verica Aleksic¹, Neda Mimica-Dukić², Aleksandra Petrović¹, Monika Eper¹,
Petar Knežević¹

¹ Department of Biology and Ecology,

² Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences,
University of Novi Sad, Novi Sad, Serbia

*Corresponding author: petar.knezevic@dbe.uns.ac.rs

KEYWORDS: *Acinetobacter baumannii*; essential oils; mechanism of action; protein leakage

INTRODUCTION: *Acinetobacter baumannii* is a nosocomial pathogen that exhibit high level of resistance to conventional antibiotics. Essential oils are frequently examined alternative antimicrobial agents against Gram negative bacteria, including *Acinetobacter baumannii*. However, the basis of their mechanisms of action has not been fully established. Thus, the study has been made to elucidate the mode of essential oil action in Gram negative bacteria, using *A. baumannii* as a model.

OBJECTIVES: The aim of this study is to examine the involvement of essential oils in membrane disruption during the inhibition of *Acinetobacter baumannii* at sub- and super-inhibitory concentrations by studying protein leakage from cells.

METHOD / DESIGN: *A. baumannii* reference strain ATCC 19606 cells were treated with 13 different previously characterized essential oils (*Myrtus communis*, *Eucalyptus camaldulensis*, *Juniperus sabina*, *Juniperus sibirica*, *Juniperus oxycedrus*, *Juniperus phoenicea*, *Artemisia dracunculus*, *Foeniculum vulgare*, *Hissopus officinalis*, *Mentha x piperita*, *Origanum majorana*, *Satureja hortensis* and *Thymus srepillum*) using various concentrations (1/8×MIC, 1/4×MIC, 1/2×MIC, 1×MIC ili 2×MIC). The protein concentration in the culture supernatant of treated and untreated cells was determined at 595 nm using Coomassie brilliant blue G-250. The concentration of protein leakage was extrapolated from standard curve obtained using Bovine serum albumin (BSA).

RESULTS: The effect of essential oils from 13 different plant species on proteins release from the bacterial cells is shown on Fig.1. The essential oils of four species from the genus *Juniperus*, as well as the species of *A. dracunculus*, *F. vulgare* and *H. officinalis* had no significant effect on the

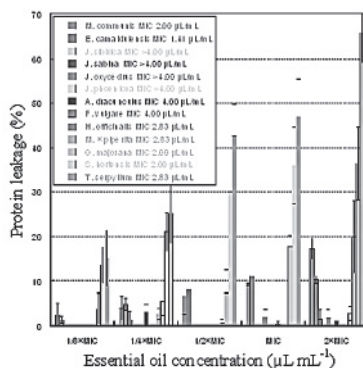


Figure 1. Essential oils effect on the *A. baumannii* protein leakage from cells

increase in the amount of released protein after treatment. Essential oil of *M. x piperita* only at concentrations of 1×MIC and 2×MIC expressed slight impact on increasing of protein quantity (3-5 % greater concentration, i.e. 22.2-22.7 mg mL⁻¹). Essential oils of the Myrtaceae plants family, *M. communis* and *E. camaldulensis*, increased protein concentration in supernatant by 7-10 % (23.6-24.11 mg mL⁻¹) with oil concentrations 1/2×MIC and 1×MIC, while with a concentration of 2×MIC increase was 10-18 %, i.e. 24.62-25.91 mg mL⁻¹ of released proteins. More significant increase of released proteins, at concentrations of 1×MIC and 2×MIC was detected for essential oils of *O. majorana* (protein increase in supernatant 19-22 %, or 22.97-26.64 mg mL⁻¹ of released proteins), *S. hotrensis* (37-38 % or 25.22-30.27 mg mL⁻¹) and *T. serphyllum* (48-65 %, which is 25.56 to 36.44 mg mL⁻¹ of released proteins). A simultaneous release of cell proteins and proteases in the supernatant may be a limitation of the method, since proteases may decrease protein content in the supernatant.

CONCLUSIONS: The results indicate that cytoplasmic membrane and/or outer membrane disruption, quantified as protein leakage from the cells, is an important mode of action for at least some of the examined oils.

17 β -HSD1 INHIBITION BY 3,17-SUBSTITUTED-16,17-SECO-ESTRATRIENE DERIVATIVES

Bianka Edina Herman¹, Suzana Jovanović-Šanta², Srđan Bjedov², Mihály Szécsi¹

¹*1st Department of Medicine, University of Szeged, Korányi fasor 8-10, H6720 Szeged, Hungary*

²*University of Novi Sad, Faculty of Science, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia*

*Corresponding author: srdjan.bjedov@dh.uns.ac.rs

KEYWORDS: 17 β -HSD1 inhibitors; anti-estrogens; seco-steroids

INTRODUCTION: The human 17 β -hydroxysteroid dehydrogenase type 1 isozyme (17 β -HSD1) catalyzes predominantly the NADPH promoted stereospecific reduction of estrone to the hormonally active 17 β -estradiol (E2) in the final key step of the estrogen biosynthesis. The highest expression and activity of the isozyme can be detected in the female steroidogenic reproductive tissues such as the ovaries and the placenta. This isozyme has a major contribution to the general gonadal supply and to the circulating level of E2 in the blood. 17 β -HSD1 is also expressed and active in peripheral tissues, where it regulates the intracellular accumulation of E2 and consequently the intracrine estrogen effect.

OBJECTIVES: Investigation of 17 β -HSD1 inhibitory effect of 3,17-substituted-16,17-seco-estrone derivatives.

METHOD / DESIGN: 17 β -HSD1 activity and inhibition was investigated via *in vitro* radiosubstrate incubation method in the presence of NADPH cofactor. Human placental cytosol was applied as enzyme source and a thin layer chromatography method was used for the isolation of the estrone substrate and the 17 β -estradiol enzyme product.

RESULTS: Substantial inhibitory potential displayed 16,17-secoestra-1,3,5(10)-triene-3,16,17-triol (IC₅₀ = 7.7 μ M). The investigated diverse 3-hydroxy-16,17-secoestra 16-nitrile compounds exerted weaker inhibition (IC₅₀ > 10 μ M). Inhibitory effect of the 3-hydroxy compounds was found stronger than that of their 3-benzyloxy counterparts.

CONCLUSIONS: These results may provide new data for the development of new 17 β -HSD1 inhibitor drug compounds. The suppressed 17 β -estradiol biosynthesis can be applied in the pharmacotherapy of estrogen-dependent diseases and gynecological malignancies.

CHEMICAL ANALYSIS AND FUMIGANT TOXICITY OF SEVERAL LABIATAE ESSENTIAL OILS TOWARDS *SITOPHILUS ORYZAE*

Aikaterini Koutsaviti¹, Vasiliki Antonopoulou¹, Anthi Vlassi¹, Spyridon Antonatos², Antonios Michaelakis², Dimitrios P. Papachristos², **Olga Tzakou**¹

¹ Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zographou, 157 71 Athens, Greece,

² Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 S. Delta Str. 14561 Kifissia Athens, Greece

*Corresponding author: tzakou@pharm.uoa.gr

KEYWORDS: Labiatae; Essential oils; *Sitophilus oryzae*; Fumigant toxicity.

INTRODUCTION: One of the most destructive insect pests of stored grain is the rice weevil, *Sitophilus oryzae* (L.). A variety of methods is applied by grain industries, in order to minimize infestation but they generally rely on use of fumigants such as phosphine. However, their usefulness is severely limited by their adverse effects on the environment and nontarget organisms and by the development of resistance. Within the framework of finding new fumigants, less toxic and environmental friendly, in comparison to conventional fumigants, natural products and essential oils are considered to be alternative sources of potentially active compounds.

OBJECTIVES: The aim of the present study was the chemical analysis and evaluation of the fumigant toxicity against the rice weevil of the essential oils obtained from the following Labiatae species: *Salvia fruticosa*, *S. pomifera* subsp. *calycina*, *S. officinalis*, *S. microphylla*, *Teucrium capitatum* and *Thymbra capitata*.

METHOD / DESIGN: Fresh aerial parts of cultivated *S. fruticosa*, *S. officinalis*, *S. microphylla* and *T. capitatum*, wild growing *S. fruticosa*, *S. pomifera* subsp. *calycina*, and *Th. capitata* were subjected separately to hydrodistillation for 3 hours and the oils obtained were analyzed by means of GC-FID and GC-MS. For the evaluation of the fumigant toxicity, 20 rice weevil adults were placed into gas-tight glass jars of 720 ml volume with metal screw caps end exposed for 48 h to the essential oils vapors. Four to six doses were tested for each essential oil and each dose was repeated four times.

RESULTS: Significant differences in the fumigant potency and the chemical composition among the essential oils were observed. *Th. capitata* exhibited the highest fumigant toxicity ($LC_{50}=3.4 \mu\text{L/L}$), and was characterized by the dominance of carvacrol (74.3%) followed by α -terpinene in significantly lower amount (7.1%). *S. pomifera* subsp. *calycina* came second in order ($LC_{50}=4.4 \mu\text{L/L}$), with *trans*- and *cis*-thujone (47.6% and 15.9%) dominating the total oil, followed by 1,8-cineole (12.0%). The volatiles of wild grown and cultivated *S. fruticosa* displayed a rather moderate toxicity ($LC_{50}=7.4 \mu\text{L/L}$ and $15.5 \mu\text{L/L}$ respectively). The major metabolite of the wild growing *S. fruticosa* essential oil was 1,8-cineole (56.3%), followed by β -pinene (7.8%), while

in the cultivated one the oxygenated monoterpenes camphor and 1,8-cineole (18.6% and 16.6%, respectively) were the most abundant components. Likewise *S. officinalis* presented a moderate fumigant potency ($LC_{50}=9.9 \mu\text{L/L}$), with *cis*- and *trans*-thujone (19.3% and 13.2%), along with viridiflorol (11.0%), camphor (9.3%) and 1,8-cineole (9.2%) characterizing the sample oil. *T. capitatum* and *S. microphylla* showed no significant fumigant toxicity ($LC_{50}>30 \mu\text{L/L}$); their essential oils contained lower levels of oxygenated monoterpenes compared to the most toxic essential oils. The monoterpene hydrocarbons α - and β -pinene (14.8%, 12.8%), followed by the sesquiterpenes β -caryophyllene (11.7%) and *epi*- α -cadinol (7.7%) were the most important volatiles of *T. capitatum* essential oil, while *S. microphylla* oil was characterized by the presence of the sesquiterpenes γ -eudesmol (20.5%), β -caryophyllene (13.7%), α -eudesmol (8.2%) and bornyl acetate (6.8%).

CONCLUSIONS: Seven Labiatae essential oils were analyzed and tested on their fumigant toxicity against adults of *S. oryzae*. *Th. capitata* and *S. pomifera* subsp. *calycina* oils exhibited the most effective fumigant activity suggesting their possible utilization as fumigants in the protection of stored products in storehouses. All the efficient essential oils against the rice weevil were rich in oxygenated monoterpenes.

CHEMICAL COMPOSITION OF *JUNIPERUS* SPP. ESSENTIAL OILS AND BIOLOGICAL EFFECTS ON THE CHORIALLANTOIC MEMBRANE (CAM) ASSAY

Aikaterini Koutsaviti¹, Enza Maria Galati², Maria Paola Germanò², Giovanna Certo²,
Olga Tzakou¹

¹ Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zographou, 157 71 Athens, Greece

² Chibiofaram Department-University of Messina

*Corresponding author: tzakou@pharm.uoa.gr

KEYWORDS: *Juniperus*; female cones; Essential oil; CAM.

INTRODUCTION: The genus *Juniperus* (Cupressaceae) comprises of 50 species, distributed throughout the northern hemisphere to tropical Africa mountains and West Indies. *Juniperus communis* L. female berry-like cones, also known as juniper berries, are most common and widely used in phytomedicine, in cosmetics and as a flavor component. Juniper berry oil is considered to have diuretic, gastrointestinal antiseptic properties; in cosmetics it is used as a fragrance element in soaps and creams, as well as in lotions and perfumes.

OBJECTIVES: The aim of the present study was: the analysis of the essential oils from *J. phoenicea* and *J. drupacea* female cones, collected in Greece; the investigation of their potential anti-angiogenic activity and the evaluation of their safety toward irritation and toxicity.

METHOD / DESIGN: Fresh samples were subjected separately to hydrodistillation for 3 hours and the oils obtained were analyzed by means of GC-FID and GC-MS. The assessment of the anti-angiogenic activity of the samples including the lack of irritant effects was performed using the CAM (chorioallantoic membrane) assay.

RESULTS: Mainly quantitative differences among the samples were observed. Limonene was the most abundant compound in *J. drupacea* (27.0%) as compared to *J. phoenicea* oil (1.6%); the content of α -pinene was high in both essential oils (*J. phoenicea* 22.1%, *J. drupacea* 26.1%), followed by germacrene D (7.4% and 7.1%, respectively). Nevertheless, qualitative differences were also detected as the diterpene 4-*epi*-abietal was present in a considerable amount (13.2%) in *J. phoenicea* essential oil, while it was not detected in *J. drupacea* sample oil.

According to our results *J. phoenicea* essential oil possesses a rather weak anti-angiogenic effect compared to the standard retinoic acid. The essential oil of *J. drupacea* showed no anti-angiogenic effect. Both essential oils showed no irritant effects at the tested concentrations, as compared to the standard sodium dodecyl sulfate.

CONCLUSIONS: The essential oils from female cones of two *Juniperus* species were investigated in respect to their chemical composition and the evaluation of their anti-

angiogenic activity. Despite the quantitative differences on limonene content, both oils were characterized by the occurrence of α -pinene and germacrene D, as main metabolites. Significant qualitative difference was the presence of 4-*epi*-abietal in *J. phoenicea* essential oil. On the CAM assay, *J. phoenicea* exhibited weak antiangiogenic effect, while *J. drupacea* oil was inactive. It is important to underline that the lack of irritant effects suggesting a safe handling of the two essential oils.

LC-DAD-MS/MS CHARACTERIZATION OF LIGNANS FROM *ANTHRISCUS SYLVESTRIS* ROOT EXTRACTS

Dejan Orčić¹, Sanja Berić¹, **Neda Mimica-Dukić¹**

¹ University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

*Corresponding author: neda.mimica-dukic@dh.uns.ac.rsddd

KEYWORDS: *Anthriscus sylvestris*; lignans; LC-MS/MS

INTRODUCTION: *Anthriscus sylvestris* (L.) Hoffm. (wild chervil) is a biennial plant from Apiaceae family, that is widespread in Europe, western Asia and northern Africa, and also started spreading through America. It is usually considered a noxious and invasive weed. *A. sylvestris* has a very limited use as a food (in Japan) and as a traditional medicinal plant (in Korea, China, Japan and Serbia), while it is not used in official medicine. However, recently revived interest in this plant resulted in several publications demonstrating high content of aryltetraline and dibenzobutyrolactone lignans with extremely potent antiproliferative activity, with IC₅₀ ranging from sub-ng/mL to µg/mL, and antiinflammatory activity. Thus, this under-appreciated plant species has a great potential as a source of bioactive natural products that could be used either directly or as precursors of semi-synthetic derivatives. In this manner, it could replace *Podophyllum* species that became endangered due to over-exploitation.

OBJECTIVES: The objective of the undertaken study was to evaluate *A. sylvestris* specimens wild-growing at Fruška Gora mountain in Serbia as potential sources of wide range of structurally diverse lignans

METHOD / DESIGN: Air-dried roots of *A. sylvestris* specimens (492 g) were extracted with methanol using percolation technique (5 cycles, ~1 L of solvent per cycle). To obtain a lignan-enriched fraction, the raw extract (75.4 g) was purified by liquid-liquid extraction. Bulk polar components (sugars, phenolic acids) were removed by partitioning in water-chloroform system. The obtained chloroform fraction (15.1 g) was subsequently partitioned in acetonitrile-hexane system, to remove highly nonpolar constituents, yielding 13.5 g (2.75 % of drug, 18.0 % of raw extract). Raw extract and all fractions were analyzed by LC-DAD-MS/MS, using Agilent Technologies series 1200 HPLC with Agilent Technologies series 6410A ESI-QqQ MS/MS. The components were separated on Zorbax Eclipse XDB-C18 column (50 mm × 4.6 mm, 1.8 µm) held at 50 °C. Mobile phase, consisting of 0.05 % HCOOH (A) and MeOH (B), was delivered in gradient mode (0 min 30 % B, 6 min 70 %, 9–12 min 100 %, post time 3 min), with flow rate of 1 mL/min. The eluted components were detected using DAD (monitoring continuous spectrum in 190–700 nm range) and MS (in both positive and negative mode, monitoring *m/z* range of 120–800). For the main detected lignans, MS² spectra of [M+H]⁺ ions were recorded at collision energies in 5–35 V range.

RESULTS: Using LC-DAD-MS/MS, it was possible to detect nearly 30 lignan compounds. The dominant peaks in chromatograms of lignan-enriched fractions correspond to dibenzobutyrolactones – yatein (saturated) and nemerosin (unsaturated). In addition, prominent peaks of several other lignans were detected – deoxypodophyllotoxin, chaerophyllin, 4,4'-dimethylmatairesinol, alongside a number of minor components – sylvestrin (*Z*-isomer of nemerosin), podophyllotoxin and bursehernin. Additionally, by interpretation of UV and MS² spectra, it was possible to tentatively identify several lignans previously not reported:

- 7-oxo-chaerophyllin (Mw=382, characterized by UV maximum at 320 nm, and [C+H]⁺ ion at *m/z* 245, accompanied by expected losses of CO and CO₂),
- hydroxy-methylenedioxy-dimethoxy-lignano-9,9'-lactone (abundant, Mw=386, absorption maximum at 284 nm, fragments at *m/z* 135 and 151),
- 7',8'-didehydrodimethylmatairesinol (Mw=384, characterized by [C+H]⁺ at *m/z* 247, accompanied by expected losses of CO and CO₂, and [A']⁺ at *m/z* 151),
- podophyllotoxone and isomers (Mw=412, absorption maxima at 280–285 nm and 321–328 nm, [C+H]⁺ fragment at *m/z* 245, accompanied by losses of CO and CO₂).

CONCLUSIONS: *Anthriscus sylvestris* was found to be a rich source of diverse lignans. By use of percolation, it is possible to extract these compounds using minimal amounts of solvent. By employing liquid-liquid extraction, it was possible to efficiently separate lignans from majority of polar metabolites (comprising ~74 % of total extract) and some of highly lipophylic constituents. Nearly 30 lignans were detected in the enriched extract, at least 13 of them fully or partially identified.

CYTOTOXIC POTENTIAL OF SELECTED COMMERCIAL HERBAL TEAS ACCORDING TO THE BRINE SHRIMP LETHALITY ASSAY

Blagica Jovanova¹, **Tatjana Kadifkova Panovska**¹ and Svetlana Kulevanova²

¹ Faculty of Pharmacy, Institute of Applied Biochemistry, Ss. Cyril and Methodius University, Majka Tereza 47, Skopje, Republic of Macedonia

² Faculty of Pharmacy, Institute of Pharmacognosy, Ss. Cyril and Methodius University, Majka Tereza 47, Skopje, Republic of Macedonia

*Corresponding author: blagicajovanova@hotmail.com

KEYWORDS: herbal teas; cytotoxic potential; *Artemia salina*; LC₅₀.

INTRODUCTION: Many commercial herbal teas are widely used for the treatment of viral infections, inflammatory processes in the urinary tract, muscle pains and spasms, respiratory diseases, rheumatism, etc. The medicinal benefits come from the plethora of phytochemical compounds they contain. Along their well-known medicinal properties, some plant species also possess toxic activity.

OBJECTIVES: The aim of our study is to determine the toxic potential of 6 commercial herbal teas: *Arctostaphylos uva-ursi* leaves, *Tussilago farfara* leaves, *Rosmarinus officinalis* leaves, *Helichrysum arenarium* flowers, *Sambucus nigra* flowers, and *Juniperus communis* berries.

METHOD / DESIGN: The *Artemia* larvae were exposed to methanol plant extracts using the Brine shrimp lethality assay, according to the procedure of McLaughlin *et al.* with slight modifications. The extracts were freeze dried, and the lyophilisates were reconstituted with DMSO (dimethylsulfoxide) in a concentration range of 0.01, 0.1, 1, 3, 5 and 10 mg/mL. After 24 hours of exposure to the plant extracts, the mortality of the larvae is observed. LC₅₀ values were calculated based on the percentage of mortality obtained for each plant extract using probit regression analysis. The cytotoxic potential was evaluated according to Meyer's scale and Clarkson's scale.

RESULTS: All tested extracts in this study manifested toxic effects according to both classification scales of toxicity. Highest toxic potential manifested the methanol extract of *Arctostaphylos uva-ursi* leaves (LC₅₀ 47 µg/mL). Many studies suggest that *Arctostaphylos uva-ursi* leaves contain the hydroquinone arbutin, a compound with a confirmed toxic potential. These findings indicate that arbutin is probably acting synergistically with other phytochemical bioactive compounds present in *Arctostaphylos uva-ursi*, and may contribute greatly for the toxicity of this plant. The lowest toxic potential showed the methanol extract of *Juniperus communis* berries (LC₅₀ 899 µg/mL).

The toxic potential of the tested plant extracts is decreasing in the following manner: *Arctostaphylos uva-ursi* (LC₅₀ 47 µg/mL) > *Tussilago farfara* (LC₅₀ 337 µg/mL) > *Helichrysum arenarium* (LC₅₀ 416 µg/mL) > *Rosmarinus officinalis* (LC₅₀ 745 µg/mL) > *Sambucus nigra* (LC₅₀ 772 µg/mL) > *Juniperus communis* (LC₅₀ 899 µg/mL). Addi-

tionally, the extracts were classified into subcategories according to Clarkson's scale of toxicity: *Arctostaphylos uva-ursi* - high toxic potential, *Tussilago farfara* and *Helichrysum arenarium* - medium toxic potential, while *Rosmarinus officinalis*, *Sambucus nigra* and *Juniperus communis* - low toxic potential.

CONCLUSIONS: Brine shrimp lethality assay is a relevant *in vivo* model for toxicity testing of plant extracts using *Artemia salina* larvae for the selection of herbal candidates with potential cytotoxic effects. Therefore, these results make the tested plant extracts promising natural cytotoxic agents. Further work should be carried out to characterize the active constituents responsible for the specific activity of these herbal teas. Also, additional work is necessary to elucidate the possible mechanism of action of these extracts.

ROSMARINIC ACID-RICH EXTRACT OF SUMMER SAVORY EXHIBITS STRONG ANTI-INFLAMMATORY ACTIVITY

Tatjana Boroja¹, Jelena Katanić¹, Vladimir Mihailović¹, San-Po Pan², Stefanie Nikles², Rudolf Bauer²

¹ Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia

² Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens University, Universitaetsplatz 4/1, 8010 Graz, Austria

*Corresponding author: tatjanaboroja@gmail.com (T. Boroja)

KEYWORDS: *Satureja hortensis* L.; rosmarinic acid; HPLC; cyclooxygenases; anti-inflammatory activity

INTRODUCTION: *Satureja hortensis* L. (summer savory) is an annual herb with oval leaves and pink flowers, traditionally used as muscle pain reliever and as a carminative in the treatment of intestinal disorders. Previous literature findings indicate that strong biological activity of summer savory is probably the result of the presence of phenolic compounds, such as rosmarinic acid, naringin, apigenin, and luteoline. Cyclooxygenases are enzymes involved in inflammation process. Cyclooxygenase-1 (COX-1) is normally expressed in almost all cells, while cyclooxygenase-2 (COX-2) is unexpressed under normal condition in the cells, but can be induced by inflammation stimuli. The conventional COX-2 inhibitors may lead to side effects, particularly in the gastrointestinal tract. Hence, tendency to investigate anti-inflammatory agents from plants with no undesirable effects on human health has been observed.

OBJECTIVES: The main objectives of our research were on investigation of phytochemical profile and *in vitro* anti-inflammatory activity of *S. hortensis* aerial parts methanolic extract.

METHOD / DESIGN: The cyclooxygenase-1 and cyclooxygenase-2 assays were performed to evaluate the *in vitro* anti-inflammatory activity. COX-1 and COX-2 inhibition assays were performed in a 96-well plate format with purified prostaglandin H synthase (PGHS)-1 from ram seminal vesicles for COX-1 and purified PGHS-2 from sheep placental cotyledons for COX-2. The concentration of prostaglandin E₂, the main arachidonic acid metabolite in this reaction, was determined by a competitive PGE₂ EIA kit. The possible mechanism of anti-inflammatory action was investigated by lipopolysaccharide-induced COX-2 gene expression in human leukemic monocyte cell line THP-1. The phytochemical screening was carried out using HPLC method.

RESULTS: Rosmarinic acid was found to be the major compound in the methanolic extract of summer savory (157.06 mg/g extract). HPLC analysis also confirmed the presence of small amount of hesperidin and caffeic acid (10.95 and 4.94 mg/g extract, respectively). Our results revealed that the examined extract was able to inhibit COX-1 and COX-2 activities (60.94% and

70.66%, respectively) at concentration 50 µg/mL. The result obtained for inhibition of COX-2 gene expression (19.18%) may suggest that this route could be one of the possible mechanisms of anti-inflammatory activity of summer savory.

CONCLUSIONS: The presented results showed that *S. hortensis* may have the potential to be used as anti-inflammatory agent in the treatment of inflammation-related disorders. Therefore, further investigations, including more extensive phytochemical characterization and standardization of *S. hortensis* extract, as well as isolation and identification of novel compounds, should provide more detailed information about components exhibiting anti-inflammatory activity.

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FAGOPYRI HERBA FEED SUPPLEMENTATION: INFLUENCE ON CHOLESTEROL AND BILE ACID METABOLISM IN RATS

Ivan Milanović¹, Aleksandra Mišan¹, Maja Đurendić-Brenesel², Vladimir Pilija², Đuro Vukmirović¹, Radmilo Čolović¹, Anamarija Mandić¹

¹ Institute of Food Technology – FINS, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

² Institute of Forensic Medicine, Clinical Center Vojvodina, Hajduk Veljkova 7-9, 21000 Novi Sad, Serbia

*Corresponding author: ivan.milanovic@fins.uns.ac.rs

KEYWORDS: Fagopyri herba; cholesterol; bile acids; faeces; hyperlipidemia; weight lowering effects

INTRODUCTION: *Fagopyri herba* has been used in the treatment of vascular diseases and reported to possess hypolipidemic effects.

OBJECTIVES: In attempt to elucidate the mechanism of *Fagopyri herba* antihyperlipidemic activity, the aim of this research was to find out if it inhibits cholesterol absorption and stimulates faecal excretion of bile acids.

METHOD / DESIGN: *In vivo* experiment on rats was set to consist of five groups: standard diet group (I), 5% *Fagopyri herba* supplemented standard diet group (II), high-fat diet group (III), high-fat 5% *Fagopyri herba* supplemented diet group (IV), a group eating the same diet as III group for 7 weeks and the same as group IV for 1 week (V). In our experiment, hyperlipidemia in high fat fed groups (III, IV and V) was induced by excess addition of cholesterol, Na-cholate and sunflower oil.

RESULTS: High-fat fed rats (III, IV and V group) showed significantly higher total levels of cholesterol and bile acids excreted in faeces in comparison to the control (I) and *Fagopyri herba* supplemented control (II) group. Levels of cholic, ω- and β-muricholic acid were found to be significantly higher in the faeces of *Fagopyri herba* supplemented high-fat diet groups (IV and V) in comparison with the group which received only high-fat diet (III). Referring to the significant difference in cholesterol content of the faeces between group V and III, *Fagopyri herba* addition seemed to stimulate cholesterol excretion.

CONCLUSIONS: The results of this experiment suggest that postponed *Fagopyri herba* introduction into the high fat diet can ameliorate the effects of prolonged high fat diet consumption in rats.

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ESTIMATION OF PHARMACOKINETIC PROPERTIES OF SALVINORIN A DERIVATIVES

Ivana Bugarski¹, Zorica Bulat², Simona Tatović², Jelena Ćurčić³, Nataša Milošević³

¹ Ministry of Interior of the Republic of Serbia, Police Directorate, Criminalistic Police Department, National Criminalistic-Technical Centre,

² University of Belgrade – Faculty of Pharmacy, Department of Toxicology „Akademik Danilo Soldatović”,

³ University of Novi Sad, Faculty of Medicine, Department of Pharmacy

*Corresponding author: ivanabugarski@gmail.com

KEYWORDS: *Salvia divinorum*; salvinorin A; kappa opioid agonist; QSAR/QSPR analysis

INTRODUCTION: *Salvia divinorum* is a psychoactive plant from the Lamiaceae family that has been used for religious practices and medical purposes by the Mazatec Indians of Oaxaca, Mexico to produce “mystical” experiences. The major active component of *Salvia divinorum* is the salvinorin A which produces hallucinogenic effect similar to the lysergic acid diethylamide (LSD), without any actions at the 5-HT_{2A} serotonin receptor which is molecular target for classical hallucinogens. Salvinorin A is, so far, the only known nonnitrogenous kappa opioid receptor (KOR) agonist. Because of its ability to target KOR, it may represent novel potential therapy for diseases manifested by perceptual distortions (schizophrenia, bipolar disorders, etc.), as therapeutic drugs for psychostimulant-related disorders, and as a neuroprotective agent for brain injury prevention in state of oxygen insufficiency (cardiac arrest, stroke, etc.). Lately it has been synthesized a number of semi-synthetic salvinorin A derivatives with selective KOR agonist activity, which pharmacokinetic parameters are still unknown.

OBJECTIVES: The aim of this study was to establish correlation between physico-chemical properties of nine salvinorin A derivatives and pharmacokinetic characteristics by QSAR/QSPR analysis.

METHOD / DESIGN: Software package ilab 2.0 was used for determining the main physico-chemical properties important for pharmacokinetics of the observed compounds as molecular weight (MW), total polar surface area (tPSA), number of hydrogen bond donors, number of hydrogen bond acceptors, number of rotatable bonds, lipophilicity coefficient (logP) and distribution coefficient (logD) as well as pharmacokinetic properties of the analyzed compounds as absorption constant (ka), permeability in jejunum P_{jej}, volume of distribution (V_d), plasma protein binding (PPB) affinity and permeability through blood brain barrier (logBBB).

RESULTS: Most of the observed compounds do not violate Lipinski's rule of five for good oral absorption. Only one compound violates one rule as it has MW over 500. According to Veber's rule of good oral bioavailability only one compound violates one rule having tPSA above 140 Å². Since all the compounds have P_{jej} predicted around

0.0007 cm/s and satisfactory k_a of below 0.05 min^{-1} , good oral absorption can be expected. The permeability of the observed compounds depends dominantly on the molecular size expressed as MW ($r^2=0.48$, $p=0.02$) with one outlier. Their Vd is moderate with values between 1.39 and 2.14 L/kg. The Vd can be presented as linear function of lipophilicity expressed as $\log P$ ($r^2=0.70$, $p=0.008$) with one compound as an obvious outlier. The percent for PPB varies between 91 and 98. Most of the compounds are neutral and are expected to bind for serum albumins, while two compounds with acidic groups are expected to bind for lipoproteins dominantly. Their PPB, regardless of the protein type, they bind depends dominantly on MW expressed as polynomial function ($r^2=0.64$, $p=0.02$). Permeability through blood-brain barrier should be limited according to Clark's rule of thumb because the observed compounds violate almost all rules. The ilab 2.0 software packages predict also limited permeation with most compounds having negative value or value around 0 for \log_{BBB} with only two compounds with values for \log_{BBB} 0.29 and 0.35 which are may guarantee relatively moderate permeation in the brain.

CONCLUSIONS: In silico analyzes of the salvinorin A derivatives, have indicated that they can be expected to have good oral availability. For all compounds analyzed, moderate Vd can be expected which guarantees lack of undesirable bioaccumulation and relatively high PPB affinity which allows comfortable daily dosage interval. For two compounds fair permeation in the brain was predicted which make them potential drugs in therapy of central nervous system disorders, while other can be observed as drugs in therapy of other body systems diseases which should not express undesirable side effects in the central nervous system since they do not permeate easily in the brain. Finally, the changes in pharmacokinetic properties of salvinorin A derivatives depend dominantly on their molecular size express as MW and lipophilicity expressed as $\log P$.

PROTECTIVE EFFECTS OF *FILIPENDULA ULMARIA* EXTRACTS ON CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

Jelena Katanić¹, Tatjana Boroja¹, Vladimir Mihailović¹, Sanja Matic²,
Nevena Stanković¹, Nezirina Mihović¹, Milan Mladenović¹, Vesna Stanković³

¹ Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia,

² Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia,

³ Institute of Pathology, Faculty of Medicine, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia

*Corresponding author: jkatanic@kg.ac.rs (J. Katanić)

KEYWORDS: *Filipendula ulmaria*; nephrotoxicity; cisplatin; oxidative stress; biochemical parameters

INTRODUCTION: *Filipendula ulmaria* (L.) Maxim. (Rosaceae), known as meadowsweet, is a perennial herb found in wild and cultivated habitats in Europe and Asia. Traditionally, the aerial parts and flowers of *F. ulmaria* are considered to have diuretic, antiseptic, antirheumatic, astringent, stomachic, and antacid activities. Cisplatin – *cis*-[Pt(NH₃)₂Cl₂] is inorganic complex which has been in clinical usage for cancer treatments although it causes a number of side effects, like vomiting, digestive tract disorders, and toxic effects on different organs, like kidneys, liver or testicular damage. Some of the suggested mechanisms of cisplatin-induced toxicity are generation of reactive oxygen species (ROS) and inhibition of antioxidant enzymes.

OBJECTIVES: Since the potent antioxidant activity of medicinal plants is well known, the present study investigated the protective effect of *F. ulmaria* aerial part (FUA) and root (FUR) methanolic extracts on cisplatin-induced toxicity in kidneys of Wistar rats. Evaluation of serum and tissue parameters and histopathological examination were performed to assess amelioration of toxicity.

METHOD / DESIGN: The animals were divided into 10 groups, each of 5 rats. Group I (negative control): normal saline was administered orally for 10 days and a single injection (*i.p.*) of 0.5 mL isotonic saline on the 5th day. Group II (cisplatin - positive control): normal saline was administered orally (*p.o.*) for 10 days and toxicity was induced on the 5th day of treatment, by intraperitoneal (*i.p.*) administration of a single dose of CP dissolved in normal saline (7.5 mg/kg body weight). The rats in groups III–V received the aerial part extract of *F. ulmaria* (FUA) and animals in groups VI–VIII received the root extract of *F. ulmaria* (FUR) dissolved in normal saline at the doses of 100, 200 and 400 mg/kg/day for 10 days *p.o.*, respectively. A single dose of cisplatin (7.5 mg/kg, *i.p.*) was administered to rats in III-VIII groups on the 5th day, 1 hour prior to extract dose. Monitored serum parameters of kidney damage were: total urea, creatinin, and uric acid levels. Kidney tissue markers of oxidative stress:

superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) were also determined.

RESULTS: The serum parameters of kidney function (urea, creatinine, and uric acid) showed that cisplatin exerted nephrotoxic properties. Results show that the levels of these three parameters were distinctly increased in cisplatin-treated group ($p < 0.05$) compared to the negative control. FUA extract decreased the concentration of urea and creatinine in dose-dependent manner, while FUR extract did not follow that trend. The highest dose of FUR (400 mg/kg b.w., Group VIII) resulted in a slight increase of urea, creatinine, and uric acid levels. The activities of CAT and SOD in kidneys of the rats in the cisplatin-treated group II were significantly decreased ($p < 0.05$) compared to the negative control. In relation to group II, treatment with *F. ulmaria* extracts in all concentrations increased the activities of CAT and SOD ($p < 0.05$) in a dose-dependent manner. The GSH levels in kidneys in the group treated only with cisplatin were extremely low. FUA and FUR extracts slightly and dose-dependently increased GSH levels. Both *F. ulmaria* extracts dose-dependently reduced TBARS levels in the kidneys ($p < 0.05$), compared to the effects of cisplatin treatment only. Histopathological analysis showed that kidney sections from cisplatin-treated rats had severe pathological changes. Normal control rats showed normal architecture of the kidney. In rats treated with FUA and FUR in three doses, significant reduction in tubular cell damage, interstitial and glomerular changes were observed.

CONCLUSIONS: Co-treatment with *F. ulmaria* extracts attenuated cisplatin-induced nephrotoxicity, regulated serum and tissue parameters related to oxidative stress and tissue damage, and helped to maintain tissue architecture.

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**IN VITRO ANTITUMOR AND HEPATOPROTECTIVE ACTIVITY OF
MUCUNA PRURIENS LINN. ETHYL ACETATE EXTRACT**

Jelena Desančić¹, Dragana Četojević-Simin², Oke-Oghene Philomena Akpoveso³, Prabal K Chatterjee³, Dunja Jakovljević¹, Vidosava Petrović⁴

¹ University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia,

² University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, Dr Goldmana 4, 21204 Sremska Kamenica, Serbia,

³ School of Pharmacy and Biomolecular Sciences, University of Brighton, BN2 4GB, Brighton, United Kingdom,

⁴ Dom zdravlja "Novi Sad", Bulevar Cara Lazara 75, 21000 Novi Sad, Serbia

*Corresponding author: jelenadesancic@hotmail.com

KEYWORDS: *Mucuna pruriens* Linn.; antitumor activity; hepatoprotective activity; cell lines

INTRODUCTION: Plants have always been the source of beneficial substances with medicinal potential. Medical plants are source of molecules that constitute many drugs. Therefore, it is important to explore the potential of plants and their application for medicinal purposes. *Mucuna pruriens* Linn. has found application in Ayurveda in the prophylactic treatment for various diseases and treatment of snakebites. Also, different *M. pruriens* extracts show a number of activities *in vitro* and *in vivo*, such as antioxidant, antiproliferative, prophylactic, anticataleptic, antidiabetic, hypolipidemic and antithrombotic activity.

OBJECTIVES: The main objective of this research was to determine *in vitro* antitumor and hepatoprotective activity of ethyl acetate extract of *M. pruriens* leaves in human and rat cell lines.

METHOD / DESIGN: Leaves were powdered using liquid nitrogen. Powder was soaked in ethyl acetate at 4 °C and stirred for 48 h. Extract was filtered and the solvent removed by rotatory evaporation at 45 atm pressure and 30 °C. Dried extract was re-dissolved in dimethyl sulfoxide (DMSO) and kept at 4 °C. The activity of extract was investigated in 15.62–250 µg/mL concentration range. To evaluate antitumor and hepatoprotective activity, human cervix carcinoma (HeLa), breast adenocarcinoma (MCF7), normal lung fibroblast (MRC-5) and rat hepatoma (H-4-II-E) cell lines were used. Cell growth was determined by measuring the total protein content by colorimetric sulforhodamine B assay.

RESULTS: The most pronounced antitumor activity of *Mucuna pruriens* ethyl acetate extract was observed in rat hepatoma cells (IC₅₀ = 38.40 µg/mL), while the activity in human cervix, breast and lung cells was lower and comparable (IC₅₀ = 54.83–61.08 µg/mL). Non-tumor/tumor IC₅₀ ratios for breast and cervix cancer cell lines (NT/T=1.09–1.11) further indicated low difference in the activity towards healthy and neoplastic cells. On the other hand, NT/T ratio for rat liver hepatoma showed higher

values (NT/T=1.59) indicating higher sensitivity of liver cells to *M. pruriens* ethyl acetate extract.

CONCLUSIONS: The results showed that *Mucuna pruriens* ethyl acetate extract possess high antitumor activity *in vitro* towards cervix and breast carcinoma cells. Although favorable activity towards healthy cells compared to neoplastic cells was demonstrated, high cytotoxicity towards liver cells was also noticed. Further investigations are needed to pinpoint the active principle/s of *M. pruriens* and to reduce its potential hepatotoxic effects.

**IN VITRO ANTITUMOR AND HEPATOPROTECTIVE ACTIVITY OF
MUCUNA PRURIENS LINN. ETHANOL EXTRACT**

Jelena Desančić¹, Dragana Četojević-Simin², Oke-Oghene Philomena Akpoveso³, Prabal K Chatterjee³, Vidosava Petrović⁴, Dunja Jakovljević¹

¹ Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia,

² Faculty of Medicine, University of Novi Sad, Oncology Institute of Vojvodina, Dr Goldmana 4, 21204 Sremska Kamenica, Serbia,

³ School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, BN2 4GB, United Kingdom,

⁴ Dom zdravlja "Novi Sad", Bulevar Cara Lazara 75, 21000 Novi Sad, Serbia

*Corresponding author: jelenadesancic@hotmail.com

KEYWORDS: *Mucuna pruriens* Linn.; antitumor activity; hepatoprotective activity; cell lines

INTRODUCTION: *Mucuna pruriens* Linn. (velvet bean) is widely known in Africa and Asia and its leaves are used for the treatment of various conditions and diseases such as anemia or diabetes. Aqueous extract of *M. pruriens* leaves contain polyphenols, flavonoids and tannins, while methanol extract have significant antioxidant activity. Related to its antioxidant activity, *M. pruriens* has been shown to have antiparkinson and neuroprotective effects. Also, it is known that *M. pruriens* ethanolic extract shows antiepileptic activity in albino rats.

OBJECTIVES: The main objective of this research was to determine *in vitro* antitumor and hepatoprotective activity of ethanol extract of *M. pruriens* leaves in human and rat cell lines.

METHOD / DESIGN: Leaves were powdered using liquid nitrogen. Powder was soaked in ethanol at 4 °C and stirred for 48 h. Extract was filtered and the solvent removed by rotatory evaporation at 45 atm pressure and 30 °C. Dried extract was re-dissolved in dimethyl sulfoxide (DMSO) and kept at 4 °C. Four additional dilutions were made and the activity of extract was investigated in 15.62-250 µg/mL concentration range. To evaluate antitumor and hepatoprotective activity human cervix carcinoma (HeLa), breast adenocarcinoma (MCF7), normal lung fibroblast (MRC-5) and rat hepatoma (H-4-II-E) cell lines were used. Cell growth was determined by measuring the total protein content by colorimetric sulforhodamine B assay.

RESULTS:

The activity of *Mucuna pruriens* ethanol extract in human cervix, breast and lung and rat hepatoma cells was comparable (IC₅₀ = 62.75-76.52 µg/mL). Non-tumor/tumor IC₅₀ ratios were in the range from NT/T=0.92-1.12 further indicating low difference in the activity towards healthy and neoplastic cells. Slightly higher NT/T ratio for rat liver hepatoma (NT/T=1.12) indicates mild hepatoprotective activity of *M. pruriens* ethanol extract.

CONCLUSIONS: The results showed that *Mucuna pruriens* ethanol extract possess high antitumor activity *in vitro* towards breast and cervix carcinoma cells. Favorable activity towards healthy cells compared to breast adenocarcinoma cells was demonstrated as well as mild hepatoprotective activity indicating safety of its use in nutrition. *Mucuna pruriens* ethanol extract could find the application as nutraceutical and in antitumor therapy.

EFFECTS OF METHANOLIC EXTRACT FROM *PIPER LONGUM* L. FRUITS ON UTERINE CONTRACTION IN RATS

Kitja Sawangjaroen¹

¹ Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110, THAILAND

*Corresponding author: kitja.s@psu.ac.th

KEYWORDS: *Piper longum*; long pepper; uterine relaxation; oxytocin; arachidonic acid

INTRODUCTION: *Piper longum* L. (long pepper) has been used in many Asian countries as a spice and an ingredient in traditional medicine. In Thailand, parts of this plant has also been used as remedy to relief many disorders such as stomachache, diarrhea, muscle pain, asthma, and inflammation. One of the herbal formula containing seeds of *P. nigrum* and fruits of *P. longum* has claimed to be effective to relief the symptoms of dysmenorrhea.

OBJECTIVES: This study was set up to investigate the effects of the methanolic extract of *P. longum* fruits on uterine contraction using isolated rat uterus as an experimental model.

METHOD / DESIGN:

1. Dry fruits of long pepper were ground and soaked in absolute methanol at room temperature for 5 days. The extraction was repeated 3 times. The methanolic extracts were then pooled and evaporated under rotary vacuum evaporator until dryness.
2. Female Wistar rats weighing between 200-250 gm. were pretreated with 100 µg of estradiol benzoate intraperitoneally 24 hours before experiment. On the day of experiment, 1 cm of each uterine horn was removed and placed in organ bath filled with Jalon-Ringer solution which was aerated continuously with 95% O₂ and 5% CO₂. The uterus was set up in the organ bath so that the contraction of the uterus could be recorded by force displacement transducer and Grass polygraph. At the commencement of experiment, the Jalon-Ringer solution was replaced with fresh solution or appropriate physiological solution for each stimulant.
3. The uteri were induced to contract with either oxytocin (1 mU/ml) or CaCl₂ (10⁻⁵ – 10⁻² M) or arachidonic acid (AA) (10⁻⁵ M) in the presence or absence of the extract (10⁻⁶ – 10⁻³ mg/ml). In other experiments which were done in parallel to the oxytocin-induced contraction, tetraethylammonium (K_{Ca} channel blocker) (10⁻³ M) or glibenclamide (K_{ATP} channel blocker) (10⁻⁵ M) was added to the organ bath to test their effects on the relaxing effect of the extract. Piperine (10⁻⁶ – 3x10⁻⁴ M) was also tested for its effect on the uterus precontracted with oxytocin (1 mU/ml).

RESULTS: The methanolic extract of long pepper (10^{-6} – 10^{-3} mg/ml) dose-dependently reduced the contraction of rat uterus induced by oxytocin (1 mU/ml) and CaCl_2 (10^{-5} – 10^{-2} M). Tetraethylammonium (10^{-3} M) and glibenclamide (10^{-5} M) could not antagonize the relaxing effect of the extract on the oxytocin-induced contraction uterus. Piperine (10^{-6} – 3×10^{-4} M) also cause an inhibition on the contraction induced by oxytocin (1 mU/ml). The contraction induced by AA (10^{-5} M) was also inhibited dose-dependently by the extract (10^{-6} – 10^{-3} mg/ml).

CONCLUSIONS: The above results suggest that the relaxing effect of the extract is, at least in part, due to the blockage of calcium movement across the plasma membrane. It is unlikely that the extract act through the inhibition of K_{Ca} channel or K_{ATP} channel. Piperine, an active principle of *P. longum*, has been shown to inhibit the contraction of rat uterus induced by oxytocin. From our previous study, piperine (0.03mg/ml) and diclofenac sodium (10^{-4} M) were capable to completely inhibit the uterine contraction induced by AA (10^{-5} M). The latter effect was also observed with the extract at high concentration in this study. These evidences suggest that the relaxing effect of methanolic extract of *P. longum* fruits may arise, in part, from piperine. In addition, piperine and the extract may also have an inhibitory effect on cyclooxygenase, a rate-limiting enzyme of prostaglandin biosynthetic pathway from arachidonic acid. The precise mechanism of action of the extract should be further elucidated.

THE FUNGICIDE PYRIMETHANIL CAUSES TESTICULAR ALTERATIONS AND SPERMATOTOXICITY IN ADULT MALE SPRAGUE-DAWLEY RATS

Margareta Nikolić¹, Milena Aleksić¹, Andrea Žabar Popović¹,
Jelena Conić¹, Perica Vasiljević¹

¹ *Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, Višegradska 33, Niš, Serbia*

*Corresponding author: nmara27@gmail.com

KEYWORDS: Fungicide; pyrimethanil; reproductive; toxicology; spermatogenesis;

INTRODUCTION: It is known that fungicides show large variations in their potential to cause toxic effects in animals, arising from the use of fungicides in agriculture and fruit production. Pyrimethanil (4, 6-Dimethyl-N-phenyl-2-pyridinamine) is an anilinopyrimidine fungicide. It belongs to organic group of systemic fungicides, which act as a contact, surface fungicide or it is moving translaminar through the plant. Pyrimethanil is preventing plant diseases caused by a broad spectrum of fungi. Concentrated suspension of pyrimethanil (400 g/l) Pyrus 400 SC, used in this study, is commercially used for suppression of gray mold of grapevine (*Botrytis cinerea*), sooty leaf spot in vines and raspberry (*Rubus* sp.) and apple scab (*Venturia inaequalis*).

OBJECTIVES: Based on previous findings that fungicides have toxic effects on the male reproductive system, and the fact that pyrimethanil has great commercial use in fruit production and vineyards, it is important to examine what are the consequences of use of this fungicide on the reproductive system in mammals, which are still unknown. Therefore this study was designed to investigate spermatotoxic effects and the ability of this fungicide to induce morphological changes in seminiferous tubules of testes in Sprague-Dawley rats treated orally with different doses of pyrimethanil.

METHOD / DESIGN: This study was designed to analyze and define the effects of 5-day oral administration of six different concentration of concentrated pyrimethanil suspension (Pyrus 400 g/l) on adult, 12 weeks old, male Sprague-Dawley rats, randomly divided into seven groups (3 per cage), maintained at 22±2°C, at 12/12h light and dark cycle. Food and water were provided ad libitum. In six experimental groups rats were orally treated with Pyrus dissolved in PBS at dosages of 500mg/kg, 600mg/kg, 700mg/kg, 800mg/kg, 900mg/kg and 1g/kg, while control group was treated only with PBS. After 5-day treatment, rats were anesthetized with 0, 3% 10% ketamidol. Immediately after sacrifice, epididymis of each animal was used for determination of motility of spermatozoa. After excision, testes were fixed in 4% paraformaldehyde and sectioned at 2µm after paraffin embedding. Sections were stained with hematoxylin and eosin and then examined on light-microscope (Leica DM2500) for analyses of different morphometric characteristics: number of spermatogenic and Sertoli cells/in the field of view, cell area and nucleus area, luminal area of seminiferous tubules and

also area of intercellular space between spermatogenic cells. Statistical analysis was performed using the statistical software package “SYSTAT13”.

RESULTS: After treatment, results showed that sperm motility was absent in 700mg/kg, 800mg/kg, 900mg/kg and 1g/kg groups, while it was present in control group and groups with lower concentrations of pyrimethanil applied. Statistical analysis of different morphometric characteristic showed that *in vivo* treatment with pyrimethanil reduce level of spermatogenic cells in the seminiferous tubules in dose-dependent manner. There was a significant reduction in the number of secondary spermatocytes from 100.067 ± 18.24 cells/field of view, after treatment with the highest concentration of pyrimethanil (1g/kg), compared to control group with 13.333 ± 11.454 cells/field of view. Also pyrimethanil caused a significant decrease in the area of spermatogenic and Sertoli cells and decrease in their nucleus area, gradually from control group to 1g/kg group. It was obtained significant decrease in area of Sertoli cells from $132.703 \pm 30.022 \mu\text{m}^2$ in control group to $63.641 \pm 8.666 \mu\text{m}^2$ in 1g/kg group. There were morphological changes of seminiferous tubules. The results showed a reduction in the luminal area, where effect of the highest concentration (1g/kg) was drastic reduction of $8,831.718 \pm 2,385.364 \mu\text{m}^2$ compared to control group with luminal area $17,356.616 \pm 5,972.545 \mu\text{m}^2$. The opposite effect was significant increase of intercellular space between spermatogenic cells in dose-dependent manner, from the 500mg/ml group to the group treated with highest concentration of 1g/ml.

CONCLUSIONS: In this experiment, results showed that pyrimethanil caused large number of pathological changes in the testes. First of all, we conclude that pyrimethanil leads to a reduction of reproductive (spermatogenic) capacity in males. The presence of necrotic cells and a significant reduction in the number of the cells indicate a proapoptotic effect of this fungicide. There was atrophy of seminiferous tubules, and the possible penetration and accumulation of fungicide in the interior of the tubules. In addition, there were observed cases of germ cells dissociation from the base of seminiferous tubules and their accumulation within the lumen. Based on previous research, conclude is that there may be ability of pyrimethanil to affect the function of Sertoli cells cytoskeleton, which impairs the adhesion of immature germ cells and Sertoli cells, which led to premature separation.

EVALUATION OF CONSUMERS STRUCTURE AND THEIR OPINION ON HERBAL REMEDIES IN THE WESTERN PART OF THE CITY OF ZAGREB

Ivana Baričević^{1,2}, Živka Juričić¹, Biljana Božin³, **Marijana Zovko Končić**¹

¹ Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovacica 1, 10000 Zagreb, Croatia

² Ljekarne Baričević, Ustanova za ljekarničku djelatnost, Zaharova 7, 10000 Zagreb, Croatia

³ Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

*Corresponding author: Marijana Zovko Končić mzovko@pharma.hr

KEYWORDS: Herbal Medicinal Products; Herbal food supplements; Consumers' opinion

INTRODUCTION: Herbal preparations are the oldest way of treating various diseases. However, their regulation and place in modern healthcare system may be somewhat confusing for the users.

OBJECTIVES: The aim of this work was to investigate the consumption of herbal products, structure of their users, as well as to examine the opinion of patients on the safety and efficacy of herbal products, the knowledge of their indications, interactions and side effects. Furthermore, the aim was to examine the users' sources of information on herbal products, which of them they consider reliable and if they receive pharmacist's instructions on the safe use of these products during the purchase.

METHOD / DESIGN: The survey was performed in three pharmacies in Zagreb. It was completed by 100 subjects, right after they have purchased an herbal product, regardless if it was food supplement or herbal drug. The questions referred to sex, age and education of users, their opinions on safety and efficacy of herbal products, as well as sources of information that lead to the purchase of the product.

RESULTS: Among users of herbal products, there were slightly more woman than man (56 vs. 44%) and majority of them were over 45 years old (59%) who finished secondary (49%) or had higher education (46%). Regarding the efficacy of the products, they were considered appropriate for treatment of serious diseases (55%), minor ailments (55%), as well as for the use by children (46%). The price played a high role in decision on purchasing a herbal product (66%). Most participants were of opinion that herbal products do not have potential for side-effects or interactions with conventional or other herbal drugs and 86% used both types of products concomitantly. Interestingly, most participants did not think that dosing is important when using a herbal product. The participants considered pharmacists (76%) and doctors (73%) the most reliable source of information on herbal products. The importance of healthy lifestyle was considered to support the activity of herbal products by a large majority of patients (89%).

CONCLUSIONS: The results point to a patients' lack of information on herbal preparations including their possible interactions and side effects. Furthermore, it was

shown that users have great confidence in the pharmacist as a source of reliable information about the activity and potential side effects of herbal preparations. This means that the pharmacist plays an important role in informing patients about taking herbal products, particularly in combination with conventional medicines, to minimize the possibility of undesired and harmful effects.

ANTIDEPRESSANT POTENTIAL OF COMMON VERVAIN (*VERBENA OFFICINALIS* L., VERBENACEAE)

Nebojša Lasica¹, Isidora Samojlik¹, Vesna Mijatović¹

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology, Novi Sad, Serbia

*Corresponding author: nebojsa.lasica@gmail.com

KEYWORDS: *Verbena officinalis*; verbascoside; antidepressant potential; psychopharmacology

INTRODUCTION: *Verbena officinalis* is a plant often used in folk medicine. It contains vast number of compounds. It has anti-inflammatory, antibacterial and antifungal, neuroprotective and many other effects, and that is why it has great significance and potential for management of many pathological conditions.

OBJECTIVES: The goal of this study was to investigate antidepressant effect of *V. officinalis* and its main constituent – verbascoside in laboratory animal (mice) model of depression.

METHOD / DESIGN: Aerial parts of *V. officinalis* were used for the preparation of aqueous extracts (I and II) by pouring boiled water over two different doses of plant which were calculated from recommended human doses. The dose of verbascoside, prepared as water solution, was recalculated from the dry matter left behind full evaporation of aqueous extract. Pharmacodynamic experiments were conducted on Swiss albino, sexually mature, male mice. Animals were divided into two groups, control group (animals treated with physiological saline and reference drugs – imipramine and fluoxetine) and experimental group (animals divided in subgroups according to treatment with two different doses of common vervain aqueous extract and verbascoside solution during 7 consecutive days). There were 6-8 animals per tested subgroup. Antidepressant potential of common vervain aqueous extracts (I and II) and verbascoside solution was examined with Forced Swimming Test (FST) and Tail Suspension Test (TST).

RESULTS: In control group, the immobilisation time in FST after the application of imipramine, the reference drug for the test, was shorter than their control time, setting the reference antidepressant results for the test. The immobilisation time in FST in subgroups treated with aqueous extract I, II and verbascoside solution was shorter than their control time, showing the antidepressant potential of tested substances. The immobilisation time in TST in control group, which was treated with fluoxetine, was shorter than their control time, also setting the reference antidepressant results for the test. The immobilisation time in TST in subgroups treated with aqueous extract I, II and verbascoside solution was shorter than control time, too.

CONCLUSIONS: In both FST and TST in mice the antidepressant effect of tested aqueous extracts and verbascoside solution was shown. Further research confirming psychopharmacological effects of common vervain and its constituents are needed for unequivocal conclusions and determination of clinically relevant results.

NEPHROPROTECTIVE EFFECT OF *SATUREJA HORTENSIS* L. AGAINST CISPLATIN-INDUCED TOXICITY

Tatjana Boroja¹, Vladimir Mihailović¹, Jelena Katanić¹, Gvozden Rosić², Dragica Selaković², Jovana Joksimović², Vesna Stanković³, Milan Mladenović¹, Nevena Stanković¹, Nezrina Mihović¹

¹ Department of Chemistry, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Serbia,

² Institute of Physiology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia,

³ Institute of Pathology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia

*Corresponding author: tatjanaboroja@gmail.com (T. Boroja)

KEYWORDS: *Satureja hortensis*; nephroprotective activity; cisplatin; oxidative damage; nephrotoxicity

INTRODUCTION: Despite the fact that cisplatin is one of the most effective anticancer drug, its clinical utilization is limited because of the nephrotoxicity, hepatotoxicity, and other side effects. One of the causative mechanisms of cisplatin nephrotoxicity is the generation of reactive oxygen species and/or impairment of renal antioxidant system. Our recent investigations revealed that *Satureja hortensis* L. (summer savory) extract possessed strong *in vitro* antioxidant activity. This prompted us to investigate the potential protective effect of *S. hortensis* on attenuation of cisplatin-induced nephrotoxicity.

OBJECTIVES: This study was designed to investigate the protective effect of methanolic extract of summer savory against cisplatin-induced nephrotoxicity and renal dysfunction in rodents.

METHOD / DESIGN: Experiment was done on 36 adult male Wistar rats divided randomly into six groups (n=6). The animals were received extract at three different concentrations (50, 100 and 200 mg/kg body weight), for ten days. Nephrotoxicity was induced by intraperitoneal administration of cisplatin (7.5 mg/kg b.w.) on the 5th day of the experiment. Positive (cisplatin), negative (water) and extract (200 mg/kg b.w.) controls were also analyzed. The evaluation of functional and structural alterations in the kidneys of treated rats was performed by biochemical and histopathological analyses.

RESULTS: It was observed that the cisplatin treatment induced significant elevations (p<0.05) in serum urea, uric acid and creatinine concentrations. On the other hand, the treatment with extract significantly reduced the level of serum parameters, as compared to the cisplatin group. Also, our results showed that extract at all tested concentrations provided significant increase in superoxide dismutase and catalase activities and lowered the level of thiobarbituric acid-reactive substances. No changes in

serum parameters and tissue antioxidant markers were observed in the group treated with extract only, as compared to the control group. Histopathological study revealed the normal renal architecture in the negative and extract control groups, while treatment with cisplatin induced atrophy, desquamation, and hydrophic degeneration in the tubular epithelium. Additionally, histopathological examination showed that *S. hortensis* markedly ameliorated cisplatin-induced damage in renal tissue.

CONCLUSIONS: The results of our research suggest that *S. hortensis* reduced nephrotoxicity caused by cisplatin and could be considered as prospective agent in therapy of renal disorders. Extensive further studies are necessary to explore the exact mechanism of *Satureja hortensis* in nephroprotection.

ACKNOWLEDGMENTS: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. III 43004).

THE INFLUENCE OF ESSENTIAL OIL OF CARAWAY (*CARUM CARVI* L.) AND CORIANDER (*CORIDANDRUM SATIVUM* L.) ON DRUG EFFECTS AND PHARMACOKINETICS

Isidora Samojlik¹, Vesna Mijatović¹, Stojan Petković², Biljana Božin³

¹ University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology,

² University of Novi Sad, Faculty of Medicine, Department of Forensic Medicine,

³ University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia

*Corresponding author: isidora.samojlik@mf.uns.ac.rs

KEYWORDS: herb-drug interactions; pharmacokinetics; *Carum carvi*; *Coriandrum sativum*; essential oil

INTRODUCTION: Despite the widespread use of herbal medicines, documented herb-drug interactions are sparse. Caraway and coriander are aromatic plants, widely used to flavour foods, added to fragrances and in medical preparations.

OBJECTIVES: This survey aimed to examine the influence of chronic intake of essential oils of caraway (CAeo) and coriander (COeo) on the effects and pharmacokinetics of certain drugs, in laboratory animals (mice).

METHOD / DESIGN: Before testing herb-drug interactions the calculated human equivalent doses of CAeo and COeo, prepared as emulsion for oral use, were orally applied during 5 consecutive days to the male mice divided into 3 principal groups (control and experimental groups receiving CAeo and COeo). Codeine, midazolam and pentobarbital were tested for analgesic, incoordination and sleeping effect. Acetaminophen and caffeine were applied orally and intraperitoneally (i.p.) for pharmacokinetic assays. Blood concentrations were determined by HPLC and pharmacokinetics parameters were calculated using WinNonlin v. 4.1 software.

RESULTS: The composition of essential oils determined by GC-MS confirmed carvone (78.8%) and limonene (10.1%) as main components in CAeo and linalool (74.6%) in COeo. The analgesic effect of codeine was increased after COeo treatment while the decrease in midazolam effect and the prolongation of pentobarbital sleeping time were recorded after CAeo intake. Both CAeo and COeo treatment showed statistically significant increase in C_{max}, AUC, AUC_{inf} of i.p. applied acetaminophen and in AUC, AUC_{inf}, V_d, T_{max} of i.p. administered caffeine, speaking in favour of enhanced body exposure to the drug. However, after oral application of acetaminophen in CAeo group, the pharmacokinetic data showed significant decrease comparing to control values, indicating the decline of drug presence in organism. Pharmacokinetics of orally applied caffeine was not significantly changed by both essential oils treatment.

CONCLUSIONS: The chronic intake of CAeo and COeo influences pharmacodynamics and pharmacokinetic properties of tested drugs. Further investigations of the exact pathway of this herb-drug interaction are needed, as well as the assessment of their real clinical significance.

The Ministry of Education and Science of Republic of Serbia (grants no. 172050 and 41012) supported this research.



ABSTRACTS

Track 5: Agriculture, food science and technology

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Franc Bavec

SITUATION AND TRENDS IN ORGANIC FOOD PRODUCTION

Franc Bavec¹, Martina Bavec¹

¹University of Maribor, Faculty of Agriculture and Life Sciences, 2310 Hoče/Maribor, Slovenia

*Corresponding author: franci.bavec@um.si

KEYWORDS: Organic farming; organic products; organic production; organic labeling; production

INTRODUCTION: Definition “Organic Agriculture is a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic Agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved“ IFOAM (2005) is regulated in European union (EU) by Council Regulation (EC) No. 834/2007 and the annexes to the implementing regulation (Commission Regulation (EC) No. 889/2008 and 1235/2008). The policies regarding support and development of organic production differ a lot among the EU countries and especially in developing countries.

OBJECTIVES: The objectives of this presentation is to present an overview of situation, trends and potentials in organic farming sector with special focus on comparison between the world situation and Eastern European developing countries.

METHODS: An analysis of data's and a review of the literature were done.

RESULTS: In spite of continuous ‘Pro et Contra’ discussion of organic farming the data of production showed that organic farming is practiced by approximately 2.3 million farmers, who managed organically about 43.7 million hectares in 172 countries. In EU 5.7% of the farmland is organic, in eleven countries more than 10% of the agricultural land is organic. At the world level from 2010 to 2013 the organic land increased from 36.017.570 ha to 43.091.113 ha, and in Eastern European countries like in Bulgaria increase was from 25.648 ha to 56.287 ha, in Croatia from 23.352 ha to 40.641 ha, in Romania from 182.706 ha to 288.261 ha but in Serbia decrease from 8.653 ha to 8.228 ha and Bosnia and Hercegovina from 580 to 292 ha. Sales of organic food and drink at global market represent about 80 billion US dollars in 2014. In Slovenia 8,7% (42.173 ha) of arable land is under organic farming with 3417 organic farmers, but only about 30% of consumed organic food is from Slovenian origin.

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Also scientific support of organic farming practices is increasing, but scientific papers were many times criticised from conventional oriented agriculture and from industry supported researchers. In the first model using meta-analysis 293 data sets Badgley et al. (2006) concluded that organic methods could produce enough food on a global scale per capita to sustain the current human population, and potentially even larger population, without increasing the agricultural land base. Based on 776 papers of biodiversity in organic and conventional farming systems (Rahmann 2011) may be concluded that organic farming produces more biodiversity. Bavec and Bavec (2015 a) concluded that in the future the challenge is designing more sustainable farming systems, how to maintain or enhance more provision of ecosystem services including biodiversity. More functional biodiversity based on underutilized crops and intercrops had to be developed (Bavec and Bavec, 2015 b). A few systematic reviews and meta-analyses comparing specifically the nutrient content of organic and conventionally produced food. Srednicka-Tober et al. (2016a, b) reported about higher quality parameters of organic milk and meat compared to conventional and Baranski et al. (2014) reported higher antioxidant and lower cadmium concentrations and lower incidence of pesticide residues in organically grown crops. The data shows also (Huber et al., 2011) that diets based on organic food has an important influence on human health and epidemiological study in France showed that consumers eating more organic food have healthier life style (Kesse-Guyot et al., 2013). Also biodynamic agriculture becomes a valid research as reported in the first review by Turinek et al. (2009). They described progress and priorities of research in biodynamic agriculture. Based on 8 years of experience in the project works (CHANNEL, LOVet I, LOVet II) knowledge transfer in organic sector has to be more oriented into practical education on site, and well organized production-processing-marketing chain in comparison with traditional support of agriculture.

Comparing Serbian and Slovenian situation it's clear that in both countries educational system of organic farming is well developed, also ideas and strategies for developmental and research activities were done, but there is difference in knowledge transfer and policy decisions, including financial support. The differences are also in opinions that organic production in Slovenia need to be for self-sufficient and in Serbia more or less exclusively for export.

CONCLUSIONS: However, developmental conditions of organic production, policy decisions supporting organic farming and transfer of knowledge into practice are the main key factors for increasing organic production. Lack some of them in individual countries may differ, like in Eastern Europe, where EU countries increased organic sector intensively than non-EU member countries. The trend of organic farming is to produce more niche foods from underutilized crops, more processed food according to organic standards and support ecological services, like the best production system for protecting environment.



Charassri Nualsri

SELECTION OF RUBBER CLONES FOR THE WHITE ROOT (*RIGIDOPORUS MICROPORUS* (KLOTZSCH) IMAZEKI) DISEASE TOLERANCE

Charassri Nualsri¹, Korakot Nakkanong¹ and Patimaporn Plodpai²

¹ Department of Plant Science and

² Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand, 90112

*Corresponding author: ncharass@yahoo.com

KEYWORDS: Rubber tree; rootstock; *Rigidoporus microporus*; white root rot tolerance

INTRODUCTION: The white root rot is one of the most important disease of rubber tree (*Hevea brasiliensis* Muell.Arg.) and cause economic losses to the latex industry in many countries including Thailand. Economic loss to rubber-grower households due to this disease depended mainly on the ages of rubber tree when the disease firstly infected and the intensity of such infection. The white root rot is responsible for yield losses of up to 50% in old rubber plantations. The fungal organism of the white root rot is *Rigidoporus microporus* (klotzsch) Imazeki. Infected trees show a general foliage discoloration, branches die back and the tree eventually dies. Roots infected with white root rot will develop a white or cream-colored mycelial mats covering the roots and lower portions of the plant's trunk. Control of white root disease is highly necessary to prevent it from spreading to neighbouring trees. Development of resistant or tolerant rubber rootstock clones is one of the most promising approaches to improve tolerance of rubber tree to the white root rot.

OBJECTIVES: The objective of the present study is to screen rubber rootstocks tolerance to the white root disease.

METHOD / DESIGN: *R microporus* were collected from basidiocarp and infected root at rubber plantations in southern Thailand. A total of 56 *R. microporus* were isolated and 24 isolates were selected and used in pathogenicity studies on RRIM 600 as a susceptible clone. The sequences of ITS were analyzed for isolate's identification. The screening of rubber rootstock tolerant to the white root disease in 9 rubber clone's seedlings was carried out. The experiment was arranged as Completely Randomized Design (CRD) with 4 replications, 20 seedlings per replication.

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RESULTS: Results in pathogenicity studies showed that all isolates were able to infect root and stem tissues of rubber tree. It was found that the most virulent isolate was RIG56. By ITS analysis, the sequences showed that RIG 56 identical to *R. microporus* strain RL (Accession no. KM246744.1) with 99% similarity). The screening of rubber rootstock tolerant to the white root disease in 9 rubber clone's seedlings (RRIM 623, KRS 36, PB 5/51, BPM 24, PB 235, JVP 80, GT 1, RRIM 600 and RRIT 251) was investigated. Results in 6 months after inoculation showed that PB 5/51 and PB 235 manifested high level of tolerance with survival percentage 92.50% and 83.75%. Whereas BPM 24 was found to be the most susceptible clone.

CONCLUSIONS: Based on 9 rubber clones studied, seedlings from PB5/51 and PB235 have high potential to use as rootstocks for the white root rot tolerance.

THE ACHIEVEMENTS IN RAPESEED BREEDING – A RESULT OF APPLYING MENDELIAN RULES

Ana Marjanović Jeromela¹, Aleksandar Mikić¹, Aleksandra Dimitrijević¹, Sreten Terzić¹, Milan Jocković¹, Dragana Miladinović¹, Wolfgang Friedt²

¹ *Institute of Field and Vegetable Crops, Novi Sad, Serbia*

² *Justus Liebig University, Institute of Agronomy & Plant Breeding, Giessen, Germany*

*Corresponding author: ana.jeromela@ifvcns.ns.ac.rs

KEYWORDS: breeding; Mendelian rules; oil quality; rapeseed; seed chemical composition

Rapeseed (*Brassica napus* L.) is the third plant source of edible oil and industrial material, while it is also used in animal feeding. During several past decades, intensive breeding efforts and optimising cropping systems led to a significant improvement of yield and quality of rapeseed. The most usual rapeseed breeding method is the hybridisation of selected parental genotypes, followed by pedigree, SSD or dihaploid methods since F₂ generation and with further line selection. The breeding material of later generations is tested in multilocation and perennial trials.

The achievements in breeding rapeseed are contributed by applying Mendelian rules. After one hundred and fifty years after their publication, they still represent a basis for introgressing monogenically inherited traits:

- Rule 1, related to the uniformity of F₁ generation, is fundamental for contemporarily widespread breeding rapeseed hybrids;
- Rule 2, regarding gene segregation in F₂ generation, is essential for understanding breeding rapeseed lines;
- Rule 3, considering an independent combining traits and genes and excluding linked ones, is the basis for combined breeding of diverse desirable traits.

The chief goal in rapeseed breeding is to enhance seed yield and quality. Since the latter is the pivotal one, a term *canola* was introduced to denote cultivars able to answer specific standards related to the content of erucic acid and glucosinolates, known in Serbia as 00-type rapeseed. The main components of rapeseed are oil and protein, with a content of 45% and 23% in good cultivars, respectively. Its seed also contains antinutritional factors, such as glucosinolates or phenols, that limit the use of rapeseed meal.

A significant advance in rapeseed breeding has been made by applying biotechnology methods. The rapeseed genome sequencing and increasing application of genomic selection enable solving the challenges for rapeseed breeders, such as:

- increasing oil content of more than 50%,
- modifying fatty acid content in commercial cultivars for various purposes,
- improving protein content and amino acid composition,

- enhancing meal quality, including both nutritional and antinutritional compounds, and
- increasing yield under unfavourable conditions, that is, various forms of abiotic and biotic stress.

We do owe our ability to reach all the rapeseed breeding goals to Gregor Mendel, who provided us with comprehending trait inheritance and thus made possible the method development from lines to hybrids. Their systematic application has led to improving all the economically important rapeseed traits and developing modern cultivar abundance.

In the end, it may be noteworthy that applying Mendelian rules may assist in developing primarily ornamental rapeseed cultivars, responsible for the beautiful scenery of a vast fields in bloom under a clear and blue sky, equally common for Saskatchewan in Canada, Ireland and East Anglia in UK, Burgundy in France, Serbia in the Balkan Peninsula, eastern Ukraine and southern Russia and numerous regions in China, lifting both heart and spirit of many a scientists or farmers worldwide.

BIO-EFFICACY OF CURZATE M8 (CYMOXANIL 8% + MANCOZEB 64%) AGAINST LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) OF TOMATO IN KARNATAKA**Devappa Venkatappa¹**

1 Department of Plant Pathology, College of Horticulture, UHS Campus, GKVK,- Bengaluru - 560 065, India

**Corresponding author: devappav@gmail.com*

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular vegetables grown all over the world. Globally, tomatoes are grown on an area 4,528,519 hectares with a production of 124,748,292 Mt. In India, tomato grown on- 540,000 hectares, producing 7,600,000 Mt. The leading tomato growing states are Uttar Pradesh, Karnataka, Maharashtra, Haryana, Punjab and Bihar. It is a good source of income to small and marginal farmers and contributes to the nutrition of the consumer. Late blight (*Phytophthora infestans*) is a common disease of tomato crops grown in the tropical highlands and temperate regions. Extended periods of leaf wetness from frequent rain or dew formation and cool to moderate temperatures (for example, 13–20°C) are required. Hot, dry weather stops disease development. The fungus persists on tomato and potato plants and residues, and in potato tubers. Many strains attack both tomato and potato. The spores are spread by wind and splashing rain. The lack of resistant cultivars and adolescence of old molecules and to tackle fungicide resistance problem, present study was aimed in screening the effectiveness of new molecule. All the treatments were applied thrice starting from disease appearance stage. The results of the study revealed Curzate M8- at 540, 810,-1080 and 1350 g a.i./ha, was evaluated for in two seasons against late blight of tomato cultivar Meghana. The efficacy of Curzate was compared with commonly used chemicals viz., Metalaxyl MZ and Mancozeb and an untreated control. Phytotoxicity effect of- Curzate M8 was evaluated using a single spray as per above concentrations. Curzate M8 at 1080 and 1350 g a.i., concentrations was found highly effective in reducing the disease and in increasing the yield as compared to Metalaxyl MZ ,Mancozeb and control. In a single season trial , Curzate M8 treated plots recorded a PDI ranging from 19.25 to 19.45 at 1080 and 1350 g a.i., and an yield of 180.5 to 189.6 q/ha as compared to a PDI of 55.21 and yield of 92.3 q/ha in control. Further, Curzate M8 did not cause any phytotoxicity symptoms in terms of chlorosis, necrosis, wilting, scorching, hyponasty and epinasty. Thus, Curzate M8 can be as safe and effective new molecule in managing the late blight of tomato in Karnataka state.

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OPTIMAL SYNTHESIS OF THE SOWER FOR THE PRECISE ORGANIC AGRICULTURE

Maja Čavić¹, Miodrag Zlokolica¹, Marko Penčić¹,
Milan Rackov¹, Ivan Knežević¹

¹ Faculty of Technical Sciences, Novi Sad, Serbia

*Corresponding author: scomaja@uns.ac.rs

KEYWORDS: organic agriculture; precision seeding

INTRODUCTION: Sowing (seeding) is the most important phase in crop growing. For a successful seeding process, two requirements have to be fulfilled:

- Correct depth: if sown too deep, roots will not be able to breathe. If sown too close to the surface, animals may disarrange the seeds.
- Proper distance: if plants are overcrowded, they will not get enough water, nutrients and sunlight, resulting in yield loss. If they are planted too far from each other, land is left unused.

Precision sowing is a method of sowing that involves placing seed at a precise spacing and depth. In this way amount of seed is reduced, crowding is avoided, there is no need for thinning, plants have enough space to grow efficiently. On the other side, by sowing fewer seeds, a very efficient germination and protection of the plants is required to make full use of the seeded area. The basic design of seeders and cultivators which are used in conventional agriculture, was developed 50 to 60 years ago, and is closely dependent on chemical crop treatment technology. The removal of weeds is one of the main problems with the transition from conventionally to organically grown food as it is today still done via hard manual labor.

OBJECTIVES: A new concept of precision sower will be introduced in such a way that it will allow the placement of seeds in a geometrically organized pattern, which is all overseen by the machine itself and then feedback to human operator. This pattern will then allow mechanized cultivation of a large number of rows at once, by “reading” a single row and utilizing a standardized block of knives.

METHOD / DESIGN: After detailed kinematical analysis of a sower mechanism an optimization synthesis will be performed with emphasis on the important design parameters, such as: overall dimensions, seeding distances etc. Solution will be verified through virtual prototype simulation.

RESULTS: The seeding unit of a sower is designed as a combination of two mechanisms, a less precise one that prepares the seeds - the feeding unit that takes seed from seed container and a very precise one that places them in the ground – the dispensing unit. Automatic system measures distance covered by the machine and initiates activation of the dispenser. Dispenser is mechanically operated thus enabling required preciseness.

CONCLUSIONS: The sower is robust, reliable, compact and can achieve broad interval of seeding distances. It places seed in its lowest position so “natural” seed placement is ensured (seed travels small distance at small speed from machine to soil). The preciseness of the space between seeds, made possible with a mechanical solution, as well as the seeds’ path from the machine to the ground, are tracked through an electronic system so the operator is fully informed on the degree of preciseness achieved in the seed placement.

SYNTHESIS OF THE MECHANISM FOR CONFIGURATION CHANGE OF THE SEEDBED CULTIVATOR

Ivan Knežević¹, Maja Čavić¹, Miodrag Zlokolica¹, Marko Penčić¹, Milan Rackov¹, Savo Bojić¹, Jovica Surčinski¹

¹ Faculty of Technical Sciences, Novi Sad, Serbia

*Corresponding author: ivanknezevic@uns.ac.rs

KEYWORDS: seedbed cultivator; mechanism; transport position

INTRODUCTION: When designing agricultural machinery where a working width is larger than the dimensions that exceed the legally stipulated width of the vehicle during transport, there is a problem of changing configuration of machine from transport position to working position, and vice versa. If the assembly for the changing configuration from working to transport position is realized (designed) only with hydraulic cylinder, then the machine behaves as a rigid system that does not have the ability to adapt to the land, which is acceptable only for small working widths.

OBJECTIVES: To solve this problem it is necessary to form an assembly for changing configuration of machine, which will consist of hydraulic components and mechanism mounted on machine frame. Also, for machines with larger working width (more than 6 meters), a solution for terrain tracking will be proposed.

METHOD / DESIGN: After detailed kinematical and dynamical analysis of the proposed mechanical system an optimization synthesis will be performed with emphasis on the important design parameters such as: overall dimensions, loads, cylinder parameters etc. Solution will be verified through virtual prototype simulation.

RESULTS: A new hydro-mechanical system for changing the configuration of seedbed cultivator during the transition from working to transport position is designed. The mechanism with the hydraulic cylinder is extended with two points with the addition of elastic element – spring, which allows customization of seedbed cultivator to the land conditions.

CONCLUSIONS: The assembly is robust, reliable, compact and applicable to many different machines without the need for major changes to the design.

TRACE ELEMENTS AND PESTICIDE RESIDUES IN SOILS ON ORGANIC AND CONVENTIONAL FARMS IN VOJVODINA, SERBIA

Maja Manojlović¹, Sanja Lazić¹, Ranko Čabilovski¹,
Dragana Šunjka¹, Darinka Bogdanović¹

¹ *University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia*

*Corresponding author: maja.manojlovic@polj.uns.ac.rs

KEYWORDS: total concentration of TEs; plant available concentration of TEs; herbicides; fungicides; insecticides

INTRODUCTION: Conventional agricultural production is characterized by intensification and high input of fertilizers and pesticides, which can cause both trace elements (TEs) deficiencies (due to increasing demand for one or few TEs), or high accumulation of TEs (due to unbalanced fertilization and improper use of pesticides) and increases the risk of retention and accumulation of pesticides residues in soil and environment. Although risk for accumulation of TEs and pesticides residues in soil is smaller in organic farming compared to conventional, monitoring of these soils is also important and has benefits.

OBJECTIVES: The aim of the research was to compare the impact of organic and conventional farming systems on soil quality with respect to TEs status and to evaluate the content of pesticide residues in soil.

METHOD / DESIGN: Study was carried out in the framework of the IPA project Agriculture Contribution towards Clean Environment and Healthy Food on seven pilot farms located in Srem and Bačka, the border region of Serbia. The investigation was conducted at three representative farms certified for organic production and four conventional farms, and within them 96 production fields with different history of farming practices. Total and plant-available concentration of TEs (DTPA) and pesticide residues were determined in soil samples taken from 0-30 cm depth. The concentration of TEs was measured by atomic absorption spectrometer (AAS). For the determination of pesticide residues, extraction and clean-up procedure was carried out using modified QuEChERS method, followed by analysis with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography with diode array detection (HPLC/DAD).

RESULTS: The results showed high variability in soil fertility not only between the farming systems (organic/conventional), but also within the same production system - between different locations and even between plots on the same farm. Soil samples taken from conventional farms had lower pH values and lower humus contents compared to the samples from the organic farms.

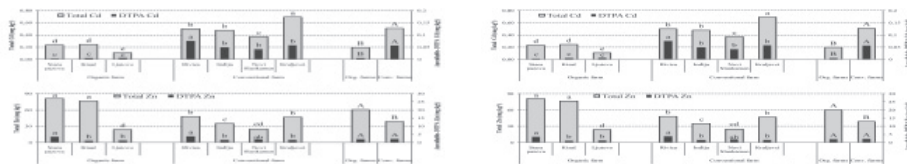


Figure 1. Total and available concentrations of trace elements in soil.

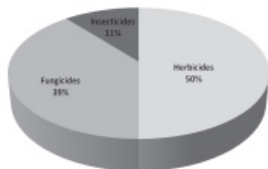


Figure 2. The proportional share of herbicides, insecticides and fungicides residues in soil samples from the conventional farms.

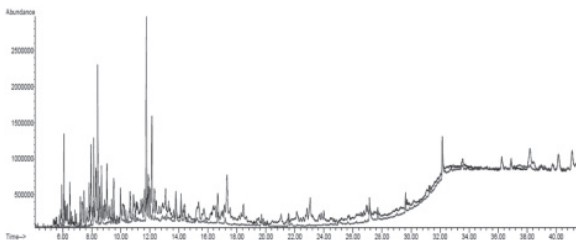


Figure 3. GC/MS chromatogram of soil sample.

CONCLUSIONS:

The results of investigation have shown that total TEs concentrations in soils are under maximum allowance values and therefore risk of TEs contamination is low. However, low level of plant available TEs, particularly zinc (Zn), is found on the most of the investigated plots on all three farms.

The results of the analysis of pesticide residues in soil samples from the conventional farms have shown the presence of sulphonylurea herbicides, metolachlor, terbuthylazine and pendimethalin, as well as the residues of some fungicides (difenoconazole, flusilazole, folpet, chlorothalonil, trifloxystrobin, epoxiconazole, pyrimethanil) and some insecticides. Soil samples originating from farms certified for organic production were pesticide free, or residues were below the LOD.

MINIATURE SOIL MOISTURE SENSOR THAT DOES NOT REQUIRE CALIBRATION

Goran Kitić¹, Vladimir Ćirić², Vesna Crnojević Bengin¹,

¹ *BioSense Institute, University of Novi Sad,*

² *Faculty of Agriculture, University of Novi Sad*

*Corresponding author: kile@uns.ac.rs

KEYWORDS: soil moisture sensor, LTCC, granular matrix

INTRODUCTION: With the current trend of world population growth and increased demand for food there is a need for efficient irrigation systems that could increase crop yield. These systems rely on accurate and reliable soil moisture sensors that can lead to average crop yield increases of 79%. In the last few decades, numerous soil moisture sensors were developed with very limited applications. A large number of existing sensor solutions based on TDR (Time Domain Reflectometry), ADR (Amplitude Domain Reflectometry) and FD (Frequency Domain) methods are limited by salinity (conductivity) of soil, which significantly complicates readout of measurement results. The common disadvantage of the above mentioned sensors is the demand for calibration according to the soil type in order to achieve desired accuracy.

OBJECTIVES: The research includes development of soil moisture sensor that is based on the phase shift method, which enables reliable measurements without the need for calibration with the respect of the soil type.

METHOD / DESIGN: The phase shift method for determination of soil moisture content relies on the fact that the water content strongly affects the permittivity of soil. Moisture content can be determined from the effective permittivity by measuring the phase shift of the electromagnetic wave that propagates along the sensor with the phase velocity. On sufficiently high frequency, above 2 GHz, the conductivity of the soil can be neglected and expression for the phase velocity can be reduced to. In this way sensor is independent of the soil type to a certain extent, since electrical conductivity of the soil is closely related to the soil type. Therefore the need for calibration of the sensor with the respect to a soil type is reduced. For the purpose of soil moisture measurement a probe was designed and fabricated, fig 1a. The probe consists of sensor element of the size 20.7 mm by 18.2 mm, made in LTCC technology that is fixed by top cover and bottom holder. Sensor element is placed in nickel plated brass cylindrical housing filled with granular matrix in the form of fine quartz sand. The granular matrix improves the contact of the sensor element and reflects moisture content of the surrounding soil.

RESULTS: Moisture measurements were performed on two soil samples originated from different soil types, at operating frequency of 2.2 GHz. Calibration curve was constructed with the help of the first sample and used to determine moisture content

of the second sample, fig 1b. Maximum absolute error was 0.01 g/g which is 5.36% of the full scale output.

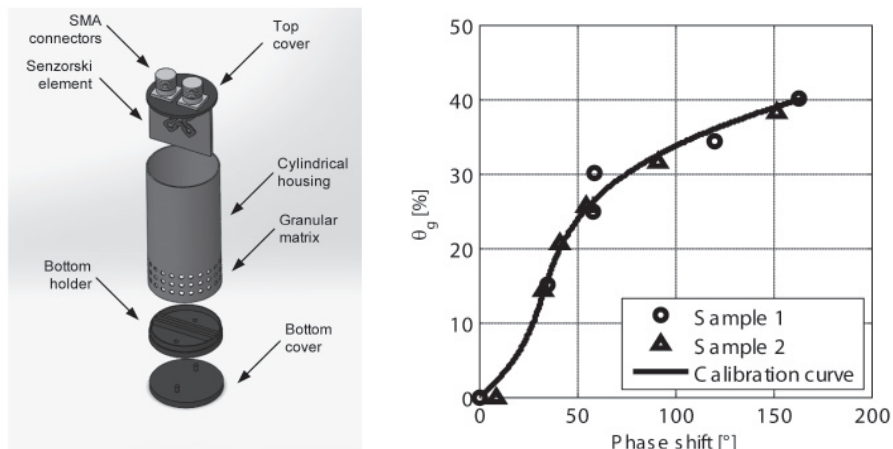


Figure 1 Miniature soil moisture sensor: a) soil moisture probe b) results of measurement of two different soil samples

CONCLUSIONS: Miniature soil moisture sensor that does not require calibration was designed and fabricated. Measurement results of two different soil samples showed good agreement with constructed calibration curve with maximum absolute error of 0.01 g/g. Sensor is made of durable elements and represent a robust, ecological and energy efficient solution convenient for integration into wireless sensor networks.

CAN SOILS OF VOJVODINA SUPPORT SUSTAINABLE INTENSIFICATION OF AGRICULTURE?**Vladimir Ćiric**¹, Ljiljana Nešić¹, Milivoj Belić¹, Pavel Benka², Dragan Radovanović¹¹ *University of Novi Sad, Faculty of Agriculture, Department for field and vegetable crops*² *University of Novi Sad, Faculty of Agriculture, Department for water management**Corresponding author: vladimir.ciric@polj.uns.ac.rs**KEYWORDS:** sustainable intensification, soil resilience, soil performance, soil quality indicators**INTRODUCTION:** The problems originated from modern land management are closely linked to global climate changes, loss of biodiversity, landscapes disturbance, as well as the deterioration soil, water and air quality. Soil is a nonrenewable resource that acts as a foundation for agricultural production and provides essential support to the ecosystem, human life and society. In order to meet the increasing food demand of a growing population on the Earth, it is necessary to maintain the level of intensification of agricultural production. As an solution in recent years the concept of sustainable intensification (SI), has appeared. This concept means increasing yields without harmful effects on the environment and further cultivation of new land.**OBJECTIVES:** The purpose of this study was to establish soil quality indicators (SQI) in 150 samples originated from different soil types in Vojvodina province (soil organic carbon, silt and clay, pH value, cation exchange capacity (CEC), soil depth and slope) and to determine the suitability of different soiltypes for of SI of agricultural production.**METHOD / DESIGN:** Six SQI for each sample were evaluated from 1-4 points. The sum of points of all SQI for a single sample classified soil in a particular category. Category 1 (6-10 points) is recommended only for extensification; category 2 has more than 10 points, and one or even several indicators are in poor condition (1 point), which suggest that intensification is possible only with high risk; category 3 (11-15 points) has a low potential for SI and intensification should be undertaken with great attention; category 4 (16-20 points) represents soils that can compensate negative impact of agriculture on the environment.**RESULTS:** According to the analyzed characteristics of the soils and their categorization, preliminarily results showed that 25% of soils is not suitable for SI, while 75% of the soils is suitable for SI. When soil was not suitable for SI, the main limiting factor was organic carbon. The top category recommended for SI includes Vertisols, Solonetz and Solonchaks, while Arenosols and Cambisols are not recommended for SI. The highest number of points has deep and heavy textured soils rich in organic carbon, such as soils in the Tisa river valley and gleyic and clayey soils of eastern and

central Banat, while the lowest score was evident in the sandy terrain and mountains. Chernozemic zone is considered as intermediate in terms of SI.

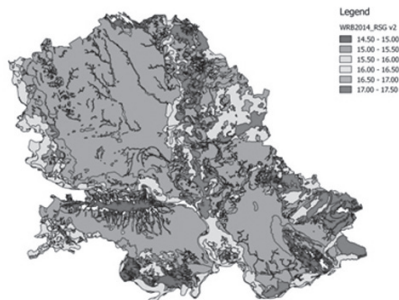


Figure 1. The preliminary map and scores of soil suitability for SI

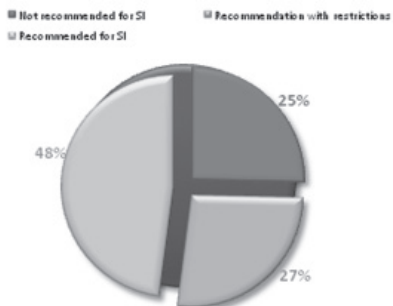


Figure 2. The relative share of soil suitability for SI

CONCLUSIONS: Six intrinsic soil properties could be successfully used for the assessment of the resilience and performance of soils in order to determine its suitability for SI. Soils of Vojvodina can support SI bearing in mind that more than $\frac{3}{4}$ of soils are classified in category 3 and 4. Fertile soils with good performances are not necessarily suitable for the SI.

THE IMPACT OF DIFFERENT ALLELES OF PHOTOPERIODIC RESPONSE GENES (*PPD*) ON AGRONOMIC PROPERTIES IN WHEAT

Ankica Kondić Špika¹, Dragana Trkulja¹, Sanja Mikić¹, Srbslav Denčić¹, Novica Mladenov¹, Simon Griffiths²

¹*Institute of Field and Vegetable Crops, Novi Sad, Serbia*

²*John Innes Centre, Norwich, UK*

*Corresponding author: ankica.spika@nsseme.com

KEYWORDS: agronomical traits, NILs, *Ppd* alleles, wheat cultivars

INTRODUCTION: Photoperiodic response is an important physiological characteristic for wheat adaptability to various environments. It is controlled by three major genes, namely, *Ppd-D1* (previously designated *Ppd1*), *Ppd-B1* (*Ppd2*) and *Ppd-A1* (*Ppd3*), located on homeologous group 2 chromosomes. Beside the photoperiodic response, these genes may have the effect on many other important agronomical traits including yield.

OBJECTIVES: The objective of this study was to identify the effect of different *Ppd* alleles on important agronomical traits (stem, peduncle and spike length) by using near-isogenic lines (NILs) of wheat cultivar Paragon in comparison to our well adapted cultivars.

METHOD / DESIGN: The experiment was carried out at the location of Rimski šančevi, Serbia (45°20' N, 19°51' E) during the growing season 2014/15.

Four sets of specific genetic material were used for the study:

Set 1 – Ten modern Serbian wheat varieties,

Set 2 – NILs of cv. Paragon for single changes in *Ppd* alleles (insensitivity and early alleles)

Set 3 – NILs of cv. Paragon for double changes in *Ppd* alleles (insensitivity and early alleles),

Set 4 – NILs of cv. Paragon for single, double or triple changes in *Ppd* alleles (null, knock-outs or late alleles)

The plot size was 2 m² (2x1) with six rows per plot in three replications. The following traits were measured: whole stem length (cm), peduncle length (cm), and spike length (cm).

RESULTS: The results have shown significant differences among the sets of the genotypes concerning analysed traits. The average whole stem length varied from 89.7 cm in the set 1 to 100.8 cm in the set 4. Significant differences regarding this trait were found between the Serbian cultivars (set 1) and the NILs from the sets 2 (98.1 cm) and 4, and between the NILs from the sets 3 (92.6 cm) and 4. The same differences among the sets of the genotypes were found for the average spike length. With respect to the average peduncle length, there was no significant difference between the Serbian

cultivars (set 1-12.4 cm) and the sets of NILs with different changes in *Ppd* alleles. The differences were only found between the NILs from the sets 2 (14.0 cm) and 3 (11.3 cm), and between the sets 3 and 4 (14.6 cm). Coefficient of variation (CV) ranged from 0.09 for whole stem length to 0.25 for peduncle length.

CONCLUSIONS: The results revealed differences between Serbian well adapted cultivars and NILs with specific combinations of *Ppd* alleles regarding whole stem length and spike length in agro-climate conditions of Southeast Europe. Peduncle length was similar in the Serbian cultivars and the NILs with different *Ppd* alleles. Changes in *Ppd* alleles in the sets 2 and 4 caused an increase of the analysed traits in relation to the cultivars developed at the IFVCNS, while the lines from the set 3 were very similar to the Serbian varieties.

ACNOWLEDGEMENT: The study was funded by the project FP7-KBBE-2011-5: “Genetics and physiology of wheat development to flowering: Tools to breed for improved adaptation and yield potential “ (ADAPTAWHEAT, project number: 289842) and by the Ministry of Education, Science and Technological Development of Serbia.

PROCESS EFFICIENCY OF CELERY ROOT OSMOTIC TREATMENT IN MOLASSES

Boško Marić^{1,2}, Vladimir Filipović¹, Milica Nićetin¹, Jelena Filipović²,
Marijana Ačanski¹, Kristian Pastor¹,

¹University of Novi Sad, Faculty of Technology,

²University of Novi Sad, Institute of Food Technology (FINS)

*Corresponding author: boskoma@yahoo.com

KEYWORDS: Celery; Osmotic treatment; sugar beet molasses

INTRODUCTION: Celery (*Apium graveolens* L.) is widely recognized as a medicinal plant and spice due to the presence of many healthful and aromatic substances. There is a growing interest in the celery, because many studies have confirmed its healing properties. Although nutritive and therapeutic valuable, celery is delicate and highly perishable plant, with high moisture content. Traditional drying processes may be inappropriate, due to the texture degradation, color alteration and nutritional loss. These disadvantages could be avoided by applying osmotic treatment (OT), which involves soaking a food, in hypertonic solution, under ambient or mild modified environment conditions. Plant is not exposed to high temperature, thus reducing sensory characteristic changes and retaining or even improving its initial nutritional value and functional properties. Furthermore, OT is environmentally acceptable and energy efficient process due to the low temperature and energy requirements and lower waste material. The complex cellular structure of plant tissue acts as a semi-permeable membrane, which allows two main counter-current flows: water outflow from the plant tissue into the osmotic solution and the simultaneous migration of solids from solution to the tissue. The type of hypertonic solution has the great influence on the kinetics of water removal and solid gain, as well as, on the nutritional and sensory quality of the final food products. Few investigations reported that sugar beet molasses is a highly effective osmotic medium for dehydration, due to the high dry matter content, and its rich chemical composition.

OBJECTIVES: Evaluation of the osmotic treatment efficiency of celery leaves in sugar beet molasses as an osmotic solution.

METHOD / DESIGN: Sugar beet molasses, concentration of 80 w/w, was used as osmotic solution. After each sampling time (1, 3 and 5 hours) celery root samples were taken out from solution, lightly washed with distilled water, gently blotted with paper to remove excessive water from the surface and weighted. Water loss (WL), solid gain (SG) and weight reduction (WR) were determined.

T5

RESULTS:

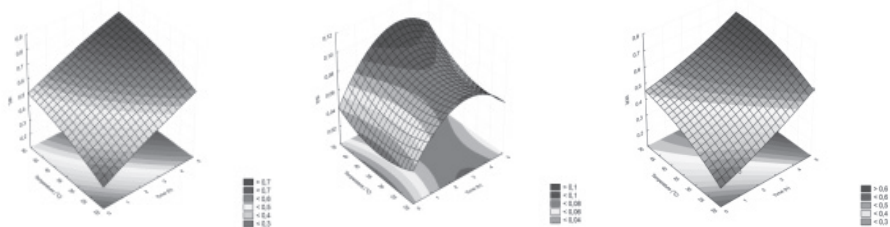


Figure 1. Dependences of WL, SG and WR from temperature and time of the osmotic treatment

From presented results it can be seen that the increase of treatment temperature has significantly influenced the increase of all three responses (WL, SG and WR). Increase of time has significantly influenced the increase of WL and WR, while SG has achieved highest values at duration of the treatment of 3 h. Mass transfer of water and solids, which is main mechanism of osmotic treatment, hence process efficiency, was intensified by increased time and temperature of the process.

CONCLUSIONS: According to the obtained results, it can be concluded that osmotic treatment in sugar beet molasses at 50°C and during 5 hours provides best process efficiency. The use of sugar beet molasses as osmotic agent is economy and environmentally reasonable, because it is a side product of the sugar industry. It can be concluded that celery root osmotically treated in molasses, with extended shelf-life and improved functional properties is suitable as food additive or functional food ingredient.

CHARACTERIZATION OF CV GRAŠEVINA WINES BY CHEMOMETRIC TECHNIQUES BASED ON THEIR MULTIELEMENT CONTENT

Sanja Peran¹, **Veronika Kubanović¹**, Renata Leder¹, Ivana Vladimira Petric¹, Mara Banović²

¹ Croatian Center of Agriculture, Food and Rural Affairs, Institute of Viticulture and Enology, Jandrićeva 42, 10000 Zagreb, Croatia

² Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

*Corresponding author: veronika.kubanovic@hcphs.hr

KEYWORDS: Graševina wine; element content; ICP-OES; viticulture zones; multivariate analysis

INTRODUCTION: Wine is a widely consumed beverage across the world and presents an obvious commercial worth. The composition of wine is influenced by many factors related to the specific production area: grape varieties, soil and climatic condition, culture, enological practices, transport and storage. In recent years, a great progress has been made in wine authentication through fingerprinting techniques, in particular in terms of provenance determination. The method combines chemical analysis, by any of a variety of instrumental analytical techniques (primarily trace element and isotope ratio analysis), and multivariate statistical analysis of the chemical data, to obtain identification and classification of wine according to geographical origin. The method assumes that the chemical composition of an agricultural product, such as wine, will reflect the composition of the provenance soil. In the case of wine, studies of this nature are being pursued in most wine producing countries. These studies focus on distinguishing wines from different countries but also some attempts were made to classify wines from different regions within one country. The smaller the geographical demarcation is, the higher are the demands on the accuracy and precision of the analytical data obtained for successful classification. *cv* Graševina (*Vitis vinifera* L.), is considered as economically the most important and the most widespread white grapevine of continental region of Croatia.

OBJECTIVES: The objective of this study was to examine the relationship of selected elements of *cv* Graševina wines in respect to their geographical origin: East Continental Croatia (Hrvatsko Podunavlje, Slavonija), West Continental Croatia (Prigorje-Bilogora, Zagorje-Međimurje) of viticulture zones B and CI, respectively. Obtained data were processed by multivariate statistical analysis to discriminate the wines according to their geographical origin.

METHOD / DESIGN: The concentration of 13 elements (Cu, Fe, Pb, Zn, K, Ca, Mg, Na, Al, Mn, Li, Rb, Sr) were determined in fifteen *cv* Graševina market ready wines originated from different Croatian sub-regions that belong to viticulture zones- B and CI by using an inductively coupled plasma optical emission spectrometer (ICP-OES).

Studied wines were discriminated using the element concentrations as chemical descriptors, and applying pattern recognition techniques, such as principal component analysis (PCA) and linear discriminant analysis (LDA).

RESULTS: According to element concentration average values, wines from sub-region Slavonija (CI) were richer in Cu, Pb, Zn, K, Ca, Mn and Sr than wines from other three sub-regions. Sub-region Podunavlje (CI) had higher concentration of following elements Fe, Mg, Na, Al and Li when compared to other sub-regions. The concentration of Rb (average 2.8 mgL^{-1}) was the highest in sub-region Međimurje-Zagorje (B) and Prigorje-Bilogora (B). Moreover, it must be emphasized that K and Mg were the elements found in highest concentrations in all wines (average concentrations 1036.3 mgL^{-1} and 98.9 mgL^{-1} , respectively). From the coefficients (loadings) of features in the first and second principal components, Sr, Mn, Al, Li, K and Fe were the dominant features in the first component, which represented 33.3% of the total variability. Pb, Zn, Cu and Ca contributed most to the second principal component, representing 19.3% of the total data variability. Defined by the first two eigenvectors, the wines from two different viticulture zones were separated in two groups (B-left side; CI-right). After applying standard LDA and according to the Wilks' Lambda values of each variable in the model, the selected most discriminating variables were Zn, K, Ca, Mg, Mn and Rb, with $p < 0.0001$. These results partially agree with the conclusions obtained by PCA, where K, Mn, Li and Sr were some of the dominant features in principal component 1, and Zn and Ca in principal component 2. By LDA analysis it was achieved a value of 96.6% of correct classification for wines that originated from viticulture zone CI and 93.3% for wines from viticulture zone B, with a significative squared Mahalanobis distance (1936.27).

CONCLUSIONS: By multivariate analysis based on the concentrations of the selected elements it was possible to discriminate *cv* Graševina wines from Croatian viticulture zones B and CI. The elements Sr, Mn, Al, Li, K and Fe were identified as suitable indicators. Wines were grouped in separate data sets to allow successful classification of wines from viticulture zones B and CI. It can be concluded that relationship between selected elements concentrations and the origin of *cv* Graševina wines was well established.

STATUS OF BUCKWHEAT (*FAGOPYRUM ESCULENTUM*) PRODUCTION WORLDWIDE

Vera Popović¹, Vladimir Sikora¹, Savo Vučković², Mladen Tatić¹, Zoran Jovović³,
Snežana Jakšić¹, Jela Ikanović²

¹ *Institute of Field and Vegetable Crops, Maxim Gorky St. 30, 21000 Novi Sad, Serbia;*

² *University of Belgrade, Faculty of Agriculture, Zemun, 11000 Belgrade, Serbia;*

³ *University of Montenegro, Biotechnical Faculty, 81000 Podgorica, Montenegro;*

*Corresponding author: bravera@eunet.rs

KEYWORDS: Buckwheat, production, yield, world.

INTRODUCTION: Buckwheat (*Fagopyrum esculentum* Möench) is an annual crop. It is not a real cereal, but its grains belong to cereals because of their similar use and chemical composition. Buckwheat is a plant predominantly cultivated for its grain, but also as a cover crop. Nowadays, it becomes a very important food in the human diet, especially in countries where special emphasis is put on the healthy food. Whole plant is utilized in medicine and grain in human and domestic animals nutrition. It is very suitable for crop rotation, because there is no usage of chemical substances for protection and therefore is suitable for organic production. In some parts of buckwheat plant there are compounds identified which have positive effects on human health.

OBJECTIVES: The main objective of this study is the analysis of buckwheat production in the world. Buckwheat is a pseudo-cereal, and pseudo-cereals are becoming increasingly important in crop production. It is a very important nutrient in the human diet, especially in countries where special emphasis is put on the healthy safe food.

METHOD / DESIGN: This paper analyses the buckwheat production parameters in the world during the period from 2012 and 2013. The research is based on the available data already existing in related statistical publications. Data from FAO 2016 were used (<http://faostat.fao.org/>).

RESULTS: Neglected arable land plants, such as buckwheat, are becoming increasingly important in crop production. Buckwheat is grown for grain, in many words for nut-like fruits. During the period 2012-2013 about average 2.44 million hectares of buckwheat was sown annually worldwide. The highest areas of buckwheat of 94.04 % in the world were in Europe and on Asian continent (1.38 million ha, 919 075 ha), that is 56.65 % and 37.75 %, respectively. Average yield of buckwheat during the monitored period was 993 kg ha⁻¹. Realized buckwheat productions in 2013 (2,547,014 t) were higher than in 2012 (2,279,877 t). Average production worldwide was 2,413,446 t and have a tendency to increase. The highest production of buckwheat of 93.67% in the world was in Europe and on Asian continent (1.33 million t, 934,741 t), that is 54.93% and 38.74%, respectively. An important production of buckwheat was in European Union from 26589 t or 11.01%. Adequate participation by continents, the most significant producers of buckwheat in the world are: Russia

(33.78%), China (29.68%), Ukraine (8.65%), France (5.38%) and Poland (3.84 %). Production of buckwheat in Serbia is concentrated on small areas.

CONCLUSIONS: Buckwheat was sown annually worldwide on 2.44 million hectares in average. In Serbia, buckwheat is cultivated in small areas, mostly in hilly-mountain areas of south-western of the country. Buckwheat breeding and technology development programme is situated at the Institute of Field and Vegetables Crops, Novi Sad, Department for alternative crops, Bački Petrovac.

Buckwheat has a multiple use. Buckwheat is frequently used as a summer cover crop because of its rapid growth and ability to out-compete weeds. Buckwheat is a good cover crop or catch crop because of its short growing season, ability to control weeds, and low maintenance. It is very suitable for crop rotation, because there is no usage of chemical substances for protection and therefore is suitable for organic production. Buckwheat grains contain a variety of nutrients, the main compounds being: proteins, polysaccharides, dietary fiber, lipids, rutin, polyphenols, micro- and macro-elements.

**EXTRACTION OF POLYPHENOLS FROM GRAPE POMACE -
METHOD DEVELOPMENT**

Veljko Čučuz¹, Jelena Cvejić¹, Ljilja Torović¹

¹*Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia*

*Corresponding author: veljkocucuz@uns.ac.rs

KEYWORDS: grape pomace; polyphenols; extraction

INTRODUCTION: *Vitis vinifera* is a grape species massively grown around the world and 80% of it is used for wine production. However, a large amount of grape pomace remaining after wine production is treated as industrial waste and can pose a problem from ecological and economical aspect. Since a significant amount of polyphenols is retained in the pomace, there are different possibilities for its revalorisation, especially in the food industry, where, due to its antimicrobial effects, can be used as natural preservative or as raw material for production of dietary supplements. Prior to using grape pomace, it is necessary to develop a method of extraction to obtain extracts with as high yield of polyphenols as possible, using solvents safe for human health.

OBJECTIVES: The objective of the study is an optimization of a method for obtaining grape pomace extracts with the highest possible content of total polyphenols and/or the highest antioxidant capacity.

METHOD / DESIGN: The development of the method for extraction of polyphenols included nine samples of grape pomace representing different grape varieties at the territory of Serbia. Since four different extraction protocols were applied, 36 samples of grape pomace extracts were obtained and subjected to the further analysis. Following drying and grinding of the pomaces, the ultrasound-assisted solid-liquid extraction was performed with four extraction mediums: 3 g/L of citric acid in water, 3 g/L of citric acid in 53% ethanol, 53% ethanol and 3 g/L of citric acid in 80% ethanol. All extractions were carried out during 30 minutes at 56°C. Antioxidant activity was determined by spectrophotometric method using reaction with DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The results were shown through IC₅₀ value which presents the concentration of extract needed to lower the starting concentration of DPPH radical by 50%. Total phenol content was determined by spectrophotometric method, using *Folin-Ciocalteu* reagent and gallic acid as the reference standard.

RESULTS: The highest total phenol content and antioxidant capacity were recorded for grape pomace extracts obtained using 53% ethanol as the extraction medium, where the antioxidant capacity in most cases (eight out of nine grape pomaces) was higher without added citric acid to ethanol, while in five out of nine samples of grape pomace the total phenol content was higher in extracts where combination of citric acid and ethanol was used. 53% ethanol proved to be a better extraction medium than 80% ethanol, while the distilled water showed the weakest potential as extraction medium.

CONCLUSIONS: Acidification of the extraction medium raises the yield of certain groups of polyphenols, but not all of them. Therefore, the difference between extracts of the same grape pomace obtained by different extraction protocols is to be expected. Furthermore, the total polyphenol content and antioxidant capacity are not in complete correlation due to antagonism between certain polyphenol groups which get involved in redox reactions. Determination of individual phenols in each of the extracts, e.g. by high performance liquid chromatography, would enable analysis where the mentioned antagonism might occur and better understanding of the results.

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THE GENETIC DIVERSITY OF MAIZE HETEROTIC GROUPS ASSESSED WITH MICROSATELLITE MARKERS

Sanja Mikić¹, Ankica Kondić-Špika¹, Dušan Stanisavljević¹, Ljiljana Brbaklić², Dragana Trkulja¹, Bojan Mitrović¹, Marina Čeran¹

¹*Institute of Field and Vegetable Crops, Novi Sad, Serbia,*

²*Research and Development Center Biogranum, Novi Sad, Serbia*

*Corresponding author: sanja.mikic@ifvcns.ns.ac.rs

KEYWORDS: inbred lines; heterotic groups; maize; microsatellites

INTRODUCTION: In order to fully exploit the phenomenon of heterosis, it is a common practice for maize breeders to classify inbred lines into heterotic groups. This classification facilitates the choice of inbred lines for crossing and obtaining hybrid vigor that leads to higher grain yield. The long-term selection during which the less favorable genotypes were being discarded, the genetic diversity of working collections gradually has declined. Today, the large percent of the commercial hybrids originates from only few reselected inbred lines.

OBJECTIVES: The aim of this study was to estimate genetic diversity of three main heterotic groups of the maize breeding programs at the Institute of Field and Vegetable Crops

METHOD / DESIGN: Sixty representative inbred lines from BSSS, Lancaster and Iodent groups were genotyped with 30 microsatellite markers. The DNA was extracted from maize seedlings, amplified in PCR and the obtained fragments were separated with capillary electrophoresis. The molecular data was analyzed in GenALEX and PowerMarker software and presented with molecular diversity parameters.

RESULTS: The largest total number of alleles (224), average number of alleles per locus (7.5), gene diversity (0.71) and number of loci unique loci for the group (58) were detected in BSSS group, following by Lancaster with the total number of alleles (172), average number of alleles per locus (5.7), gene diversity (0.66) and number of unique loci (25), whereas Iodent group showed somewhat lower values: the total number of alleles (161), average number of alleles per locus (5.4), gene diversity (0.67) and number of unique loci (16). Microsatellites *bnlg666*, *dupssr23*, *umc1083*, *dupssr10* and *umc1035* were the most efficient in differentiating between BSSS, Lancaster and Iodent lines as 6 to 11 of their alleles were unique for each heterotic group. Analysis of molecular variance showed that genetic variation was found mainly within groups (98%), while variance among three heterotic groups was only 2%.

CONCLUSIONS: The results indicate the satisfactorily level of genetic diversity of all the groups, but also the importance of the most diverse BSSS group in BSSS-Lancaster and BSSS-Iodent heterotic patterns, as well as the positive effects of previous introduction of new germplasm and reselection in existing inbred lines collections.

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ASSOCIATION ANALYSIS OF YIELD RELATED TRAITS IN MAIZE

Sanja Mikić¹, Ankica Kondić Špika¹, Brbaklić Ljiljana², Dušan Stanisavljević¹, Dragana Trkulja¹, Marina Čeran¹, Bojan Mitrović¹

¹*Institute of Field and Vegetable Crops, Novi Sad, Serbia*, ²*Research and Development Center Biogramum, Novi Sad, Serbia*

*Corresponding author: sanja.mikic@ifvcns.ns.ac.rs

KEYWORDS: associations; maize; microsatellites; yield components

INTRODUCTION: Breeding for yield is a main target in maize hybrid development. Grain yield consists of yield components, the traits that correlate and contribute to the amount of produced yield. The importance of yield components genetic analysis can be observed from a theoretical standpoint, by understanding how yield is formed, and from a practical standpoint, by applying this knowledge to improve maize yield.

OBJECTIVES: The object of this study was to identify molecular markers, which co-localize with genetic factors controlling yield related traits of maize using association analysis approach.

METHOD / DESIGN: A set of 96 maize inbred lines developed at the Institute of Field and Vegetable Crops in Novi Sad was analyzed with 40 SSR markers. Simultaneously, the phenotypic data was obtained for five yield components measured at five environments. After assessing population structure, the association analysis was performed with the general linear and mixed linear models using software Tassel.

RESULTS: The significant associations were determined between the yield-related traits and several microsatellites. For ear diameter, associations with markers *umc1025* and *phi083* were consistent in all environments. The marker *umc1122* had significant associations with number of kernel rows in three environments. Maize ear length was correlated with *bnlg1237*, *phi034* and *bnlg162* in five, four and three environments, respectively. For marker *umc1025*, significant associations with kernel number per row were detected in three environments, while 1000-kernal weight was correlated with four markers, namely *umc1122*, *umc1014*, *bnlg1792* and *bnlg162*, in at least three environments. The proportion of phenotypic variation of the yield components explained by the markers varied from 2.8 to 22.1%.

CONCLUSIONS: The different QTLs controlling different yield components indicate the complex nature of maize grain yield and the inconsistency of the associations in some environments reveals the presence of genotype × environment interaction. Despite this, the results suggest that the chromosome regions containing QTLs associated with grain yield components consistently across all environments could be useful in marker assisted selection.

ACKNOWLEDGEMENTS: This research is funded by the Ministry of Education, Science and Technological development of the Republic of Serbia.

CHALLENGES IN SUNFLOWER BREEDING FOR CHANGES IN OIL QUALITY

Sandra Cvejić¹, Siniša Jocić¹, Milan Jocković¹, Ana Marjanović Jeromela¹, Dragana Miladinović¹, Ivana Imerovski¹, Aleksandra Dimitrijević¹

¹ *Institute of Field and Vegetable Crops, Makisma Gorkog 30, Novi Sad, Serbia*

*Corresponding author: *sandra.cvejic@nsseme.com*

KEYWORDS: sunflower; oil quality; fatty acids; tocopherols

INTRODUCTION: As an industrial plant, sunflower is cultivated for oil production, so breeding effort have mainly been focused on the increase the genetic potential of seed yield and oil content in newly created hybrids. Recently the issue of oil quality has become one of the main challenges put in front of sunflower breeders. The optimal quality of sunflower oil depends on the purpose of its use, in food or non-food industry. Following the trends of the food and other industries, sunflower breeders have been able to significantly change the quality of the oil. Creating various types of oil plays a significant role in future development of sunflower as an agricultural crop.

OBJECTIVES: The objectives of the study were to determine oil quality (the fatty acids and tocopherol profiles) within the sunflower inbred lines and to classify them.

METHOD / DESIGN: Total of 25 inbred lines were developed at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Inbred lines were analysed for stearic, palmitic, oleic, linoleic, linolenic and arachidic acid content and alpha, beta, gamma and delta-tocopherol content. Classification of sunflower inbred lines was performed using cluster analysis.

RESULTS: Six sunflower inbred lines were classified as high oleic lines while remaining 19 inbred lines belonged to standard linoleic type. High oleic lines have increasing content of oleic acid (80% and more), compared to a standard linoleic type of sunflower which has about 20%. Moreover, achievements in sunflower breeding have enabled creating changes in the content and type of tocopherols in the oil. Among 19 inbred standard linoleic lines, 6 were classified with higher content of alpha tocopherol, 1 with beta and 3 with presence of gamma and delta tocopherols.

CONCLUSIONS: Identification of new inbred lines with changed oil quality will lead to the creation of hybrids that could be used for a particular purpose either in industry or in human nutrition. The oil of high-oleic sunflower has excellent nutritional properties and is a suitable raw material for many derivatives of the chemical industry and for the production of high quality biodiesel. Changes in the tocopherol type were achieved by replacing the alpha-tocopherol, which is predominantly present in sunflower oil, with beta-, gamma- and delta-tocopherols, which have a higher chemical activity making the sunflower oil more oxidative stable.

**STORAGE STABILITY OF SOUR CHERRY POMACE EXTRACT
ENCAPSULATED IN WHEY PROTEIN**

Mirjana Jakišić¹, Vesna Tumbas Šaponjac¹, Sonja Djilas¹, Jasna Čanadanović-Brunet¹, Gordana Četković¹, Jelena Vulić¹, Slađana Stajčić¹

¹ *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

**Corresponding author: jakisicmirjana@gmail.com*

KEYWORDS: sour cherry; pomace; encapsulation; whey protein; polyphenols; anti-oxidative activity

INTRODUCTION: Polyphenols have attracted the interest of many researchers and the general public due to the potential health benefits to humans. Employment of polyphenols in food, pharmaceutical and biomedical fields has emerged from biochemical and epidemiological studies confirming their numerous biological properties, including antioxidant. However, polyphenols are very sensitive to several environmental factors, such as heat and light, undergo degradation in water or oxidation, with a consequent loss in activity. Also, they show high rate of metabolism and rapid elimination from human body. Most of them are high molecular weight and cannot be easily absorbed. Techniques are developed for overcoming these drawbacks and one of them is encapsulation. This process protects the core material by encapsulating it with a wall or coating material that prevents exposure to deteriorating conditions and can promote controlled release of the encapsulate. Proteins are commonly used wall materials for encapsulation, having additionally many excellent functional properties.

OBJECTIVES: The aim of this study was to recover sour cherry pomace polyphenols and protect them by encapsulation in whey protein. Also, storage properties of the obtained encapsulate, in terms of polyphenol retention and changes in antioxidant activity, were assessed during six weeks.

METHOD / DESIGN: Sour cherry pomace, a waste remained after juice production, was extracted with food-grade solvent (50% ethanol) and encapsulated in whey proteins (core:coating ratio 2:1) by freeze-drying. Total polyphenol (TPh) and anthocyanin contents (TAc), as well as antioxidant activity (AA) were tested during six weeks of storage at room temperature. Storage indices (TPh, TAc and AA) were measured using spectrophotometrical assays (Folin-Ciocalteu, pH-single and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical assay, respectively).

RESULTS: The encapsulation efficiency of polyphenols from sour cherry pomace on whey proteins was found to be 90.10%, indicating the appropriate selection of the material and the procedure for encapsulation. Storage properties of encapsulates in terms of TPh, TAc and AA, have indicated that the retention of polyphenols after six weeks at room temperature was 67.33%, the content of anthocyanins has decreased very slightly (for 1.45%), while the decrease in antioxidant activity was 35.04%.

CONCLUSIONS: Encapsulation of natural pigments can be an interesting alternative for the replacement of artificial colourants in the food and pharmaceutical industry. Based on the results from this study showing high retention of anthocyanins in encapsulates after six weeks of storage, as well as preservation of their antioxidative potential, the reported technique can be used for obtaining quality encapsulates for their further use as food colorants and functional food additives, in addition to valorization of fruit waste.

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**OPTIMIZATION OF BEETROOT JUICE ENCAPSULATION
IN SOY PROTEIN**

Mirjana Jakišić¹, Vesna Tumbas Šaponjac¹, Sonja Djilas¹, Jasna Čanadanović-Brunet¹,
Gordana Četković¹, Jelena Vulić¹, Slađana Stajčić¹

¹ *University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

*Corresponding author: jakisicmirjana@gmail.com

KEYWORDS: beetroot juice; polyphenols; betalains; antioxidative activity; encapsulation

INTRODUCTION: Balance between the production of reactive free radical species, and the protective mechanisms against them is considered important in preserving good health. Antioxidants can attenuate the effects of free radicals *in vitro* and delay many events that contribute to cellular damage. Research has demonstrated that fruits and vegetables are a rich source of antioxidant compounds such as ascorbic acid, carotenoids, flavonoids and other phenolics. However, natural antioxidants can be sensitive to environmental or technological process conditions such as temperature, pH, oxygen, water, light, radiation, the presence of metal ions and certain enzymes. Their stabilization could be improved using encapsulation technologies. One of the widely used techniques of encapsulation is freeze-drying which additionally preserves most of the initial raw material properties such as color, flavor, texture and biological activity. The material used for the design of the protective shell of encapsulates has to be food-grade and biodegradable. Soy proteins have favorable functional properties, they are cost-effective and easily available and high nutritious which makes them potentially good carriers for bioactive compounds in food formulations.

OBJECTIVES: This work was aimed at investigation of optimal conditions for bonding, concentration and stabilization of valuable bioactive compounds from beetroot juice on soybean proteins, using freeze-drying method.

METHOD / DESIGN: Encapsulation of beetroot juice on soy proteins has been optimized in terms of encapsulation parameters, i.e. wall:core ratio, juice dilution and mixing time. Optimization was performed using response surface methodology (RSM) and Box-Behnken experimental design. The optimal conditions for obtaining the enriched soy protein with the highest encapsulation efficiency of polyphenolic compounds (EE) and the highest antioxidant activity on stable DPPH radicals (AA) have been selected. In order to confirm the optimization process, these conditions were applied to obtain the optimal sample which was further characterized in terms of the content of total polyphenolic compounds (TPh), flavonoids (TFI) and betalains (TBt), as well as AA and reducing power (RP).

RESULTS: Multi-response optimization, which considered both responses EE and AA, has indicated that the best encapsulation of beetroot juice is accomplished when soy

protein is mixed with undiluted juice for 9.8 min in ratio 50 g/l. Applying these conditions the optimal encapsulate has shown the following characteristics: TPh 75.91 mg gallic acid equivalents/100 g, TFl 12.5 mg rutin equivalents/100g, TBt 522.28 mg/100g, out of which 262.55 mg/100g betaxanthines and 259.73 mg/100g betacyanins, AA 255.49 mg Trolox equivalents/100g and RP 453.62 mg Trolox equivalents/100 g. The results of EE and AA for optimal sample were in accordance with the ones obtained by RSM optimization, with no statistical difference ($p < 0.05$), confirming the validity of optimization process.

CONCLUSIONS: Based on these results the obtained optimized conditions could be used for encapsulation of bioactive compounds from beetroot juice on soybean proteins. The resulting enriched soy protein has potential application in the food industry as food colorant and as a component of functional foods.

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ORGANIC QUINOA GREEN BIOMASS AND LEAVES QUALITY DEPENDING ON FERTILIZATION

Maja Manojlović¹, Mirela Matković¹, Milan Stojšin¹, Ranko Čabilovski¹,
Timea Hajnal-Jafari¹, Simonida Đurić¹, Dragana Stamenov¹

¹ University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

*Corresponding author: maja.manojlovic@polj.uns.ac.rs

KEYWORDS: Organic agriculture, organic fertilizers, microbial fertilizers.

INTRODUCTION: Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal, having increased importance nowadays due to its nutritional properties: presence of protein (all essential amino acids), trace elements, vitamins, linoleic acid (omega-3) and amylase, and it does not contain gluten (Demin et al., 2012). On the other hand, it is less known that green leaves can be used for human nutrition, consumed in salad, and little information is available about their quality and agricultural practices effects on the quality and the biomass, especially in organic farming.

OBJECTIVES: The aim of this preliminary research is to investigate the effect of organic and microbial fertilizers application on the organic quinoa green biomass and leaf chemical composition in conditions with and without irrigation.

METHOD / DESIGN: Two-factorial field experiment (fertilization and irrigation) with quinoa (variety KVL - 37) with four replicates per treatment was set up on the certified organic field on Chernozem in Novi Sad (Vojvodina, Serbia). Fertilization treatments were: Ø – control; N1 – 85 kg N ha⁻¹ („Siforga”); N2 – 170 kg N ha⁻¹ („Siforga”); N1+M - 85 kg N ha⁻¹ + „BactoFil B-10; M - „BactoFil B-10. „Siforga” is a organic fertilizer (N:P:K 5:3:8) on the basis of dry chicken manure and ”BactoFil B - 10” is a microbial fertilizer and contains several PGP bacteria (*Azotobacter vinelandii*; *Azospirillum lipoferum*; *Bacillus megaterium*; *Bacillus subtilis*; *Bacillus circulans*; *Pseudomonas fluorescens*).

The samples of green aboveground biomass consisted of 10 aboveground parts of quinoa plants and were taken in the phase on intensive growth, 32-day and 38-day after sowing on non-irrigated and irrigated plots, respectively (due to bad weather conditions). The concentration of total N, proteins, nitrates, Fe, Zn and Mn were determined in the leaves biomass samples.

RESULTS: Sprinkler irrigation positively affected plant biomass, fresh and dry leaves biomass. Higher dose of organic fertilizer (N2) and lower dose of organic fertilizer + microbial fertilizer (N1+M) increased the yield and protein content compared to the other treatments. However, the concentration of nitrates in FM leaves on all treatments was below the limit given by EU Commission reg. No 466/20019. The concentration of Fe, Zn, and Mn were lower on the treatments were higher biomass yield were achieved

(N2, N1+M), probably due to the dilution effect. The concentration of Zn was higher on the irrigated plots.

Table 1. Plant aboveground green biomass, fresh and dry leaves biomass (g per plant)

Treatments	Plant mass	FM leaves	DM leaves	Treatments	Plant mass	FM leaves	DM leaves
Without irrigation				With irrigation			
Ø	24.4	12.5	1.5	Ø	58.4 b	30.2 bc	2.7 b
N1	27.2	13.5	1.5	N1	57.2 b	27.6 c	2.6 b
N2	32.7	17.0	1.9	N2	78.8 ab	39.0 ab	3.4 a
N1+M	32.9	17.7	2.0	N1+M	80.5 a	39.9 a	3.1 ab
M	28.6	14.8	1.7	M	63.4 ab	32.0 bc	2.7 b

CONCLUSIONS: The application of organic fertilizers positively affected green biomass yield and leaves protein content. The results show that in the agroecological conditions in Vojvodina / Serbia application of organic and microbial fertilizers as well as irrigation are very important agricultural practices even on fertile soil. This research gave a valuable contribution to the organic production of quinoa, especially to small farmers to whom quinoa may be an interesting new agricultural crop.

FUSARIUM GRAMINEARUM AS THE CAUSAL AGENTS OF SOYBEAN SEED ROT

Maja Ignjatov¹, Dragana Milošević¹, Zorica Nikolić¹, Svetlana Balešević - Tubić¹, **Kristina Petrović¹**, Dragana Bjelić¹, Jelena Marinković¹

¹*Institute of Field and Vegetable Crops, Maksima Gorkog 30, Novi Sad, Serbia*

*Corresponding author: maja.ignjatov@nsseme.com

KEYWORDS: Soybean; *Fusarium graminearum*; seed; rot

INTRODUCTION: Soybean (*Glycine max* L. (Merr)) is one of the most important field crops in Serbia, where it is grown on about 160.000 hectares. *Fusarium* sp. on soybean seeds are a common seed-borne pathogen, which can damage seeds and seedlings by causing seed and root rot. During the seed health analysis in 2015, *Fusarium* infected seeds were recorded with the percentage of infected seeds ranging from 3% to 17%.

OBJECTIVES: The objective of this paper was isolation and identification of the causal agent of soybean seed rot from 12 seed lots, based on morphological and molecular characteristics of the pathogen.

METHOD: Soybean seeds were sterilized with 3% NaOCl for 3 minutes, dried and placed on a potato dextrose agar (PDA), and then incubated for seven days at 22±2°C. For morphological identification, fungi isolates were sub-cultured and single-spored on both PDA and Leaf carnation agar (CLA) for 7-10 days at 25°C, in alternating cycles of 12 hours light and 12 hours darkness. To obtain a DNA sequence – based identification, total DNA was extracted directly from the mycelium of the 17 isolates tested with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Following DNA extraction, the translation elongation factor of 1-*alpha* region was amplified by PCR with the primer pair EF1 (ATGGGTAAGGAGGACAAGAC) and EF2 (GGAAGTAC-CAGTGATCATGTT). The PCR mixture with a total volume of 25 µl consisted of 2 × Eppendorf Master Mix (Taq DNA polymerase 1.25 U, 30 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂; 0.1% Igepal-CA630; 0.2 mM dNTP); 1µM of each primer, and 1µl of fungal DNA. The PCR conditions were as follows: 2 min at 94°C, 35 cycles of (94°C 1 min, 53°C 1 min, 72°C 2 min), and 10 min at 72°C. Amplicons were electrophoresed in 1.5% agarose gel (Invitrogen) with ethidium bromide. Purification and sequencing of the PCR-amplified DNA fragments was done in Company MACROGEN, Seoul, South Korea (<http://dna.macrogen.com>, Korea). For sequences analysis FinchTV Version 1.4.0. were used and nucleotide sequences filed in the GenBank database under the National Center for Biotechnology Information (NCBI).

RESULTS: Typically white to reddish mycelia, showing yellow tint and pink pigment on the reverse surface of the PDA, were developed. Isolates formed hyaline, thin-walled, slightly curved, fusoid macroconidia, with 4-6 septae (28 to 60 × 2 to 2.5

µm). Polymerase chain reaction (PCR) with primers designated as EF1 and EF2 were created as choice of a single locus identification tool in *Fusarium* genus. The presence of amplicon of ~700 bp in size in all investigated was confirmed by comparing the amplified DNA fragments with the marker and positive control. Identification of one isolate (S1) was performed by sequencing the translation elongation factor (*EF-1α*) gene, which was deposited in the NCBI GenBank database under accession number KX092467. BLASTn queries of GenBank and the *Fusarium* ID-database showed 100% identity to accessions LT548550 and JX118857 from an unnamed phylogenetic species within the *Fusarium graminearum* species.

CONCLUSIONS: Based on the completion of Koch's postulates and sequence analysis, the causal agent of soybean seed rot was determined to be *Fusarium graminearum* (Schwabe), with the percentage of infected seeds ranging from 3% to 17%. Since that heterogeneity of the population of pathogens of soybean as well as their presence and harm, especially in the production in the Serbia, determining the fungal populations has multiple significance from the point of care, breeding and creating resistant lines and varieties.

THE APPLICATION OF PERICARP ANATOMICAL ANALYSIS IN QUALITY EVALUATION OF PEPPER FRUITS

Lana Zorić¹, Žarko Kevrešan², Jasna Mastilović², Zoran Ilić³,
Dunja Karanović¹, Jadranka Luković¹

¹ *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg D. Obradovića 2, 21000 Novi Sad, Serbia*

² *University of Novi Sad, Institute of Food Technology, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia*

³ *Faculty of Agriculture Priština-Lešak, Kopaonička bb, 38219 Lešak, Serbia*

*Corresponding author: lane.zoric@dbe.uns.ac.rs

KEYWORDS: pepper fruit; pericarp anatomy; mesocarp_

INTRODUCTION: Vegetable fruit anatomical structure plays an important role in their technological quality determination. Pepper fruits can be used as food in many different ways (fresh consumption, pickling, cooking drying...), and desirable quality parameters depend on their utilization. The fruits which are intended for drying and utilization of whole dried fruits, which is traditional processing in Serbia, should remain whole, with undamaged pericarp, during hidratisation and the cooking process. This demand is, mainly determined by the structural characteristics of fruit pericarp.

OBJECTIVES: In this research we performed fruit anatomical analysis of traditional Serbian pepper landraces intended for different purposes, in order to define anatomical parameters which determine their quality.

METHOD / DESIGN: The landraces were grown under the same conditions following two techniques: production from transplants and production from the seed. Cross sections of ripe fruits were hand-made. Fruit anatomical properties were determined using light microscopy. Observations and measurements of pericarp parameters were performed using Image Analyzing System Motic. Pericarp thickness and the proportions of pericarp tissues were measured and calculated, as well as the size of mesocarp cells.

RESULTS: Pepper fruit pericarp was composed of one-layered outer epidermis, 1-4 layers of collenchyma, vascular bundles and well developed parenchyma, with huge vesicular cells adjacent to inner epidermis. Quantitative differences were recorded in pericarp parameters between the landraces. Although the differences were not always statistically significant, it could be noticed that the landrace intended for drying, Nizača, had thinner pericarp compared to the other landraces, with better developed peripheral tissues, cuticle and collenchyma, and lower proportion of mesocarp. Mesocarp was the thickest and composed of the largest cells in Turšijara, the landrace intended mostly for pickling. This landrace also had the lowest proportion of mechani-

cal tissue, and therefore softer pericarp. No significant differences were recorded in anatomy of the fruits of the plants grown from transplants and the seeds.

CONCLUSIONS: Thick cuticle and collenchyma were assigned as desirable traits for peppers intended for drying, because of their contribution in preservation of fruit skin during cooking. Thick mesocarp, composed of large parenchyma cells, softer pericarp, with lower proportion of mechanical tissue, were the characteristics of fruits suitable for pickling. Planting method did not affect fruit anatomical parameters.

A GC/MS METHOD FOR SUNFLOWER, ALMOND AND LINSEED OIL DIFFERENTIATION

Kristian Pastor¹, Marijana Ačanski¹, Etelka Dimić¹, Vesna Vujasinović², Đura Vujić³, Jan Sudić⁴, Sanja Dojčinović-Vujašković⁴

¹ Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia,

² College of Professional Studies in Management and Business Communication, Mitropolita Stratimirovića 110, 21205 Sremski Karlovci, Serbia,

³ Independent Scholar, University of Novi Sad, Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia,

⁴ Institute of Public Health of Vojvodina, Futoška street 121, 21000 Novi Sad, Serbia

*Corresponding author: pastor@tf.uns.ac

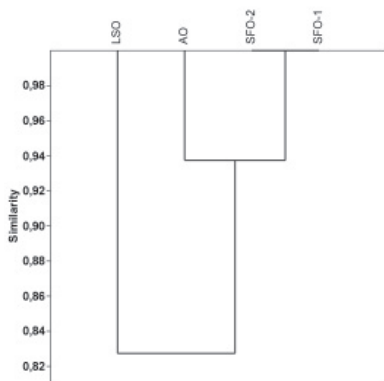
KEYWORDS: GC/MS analysis; exploratory data analysis; sunflower oil; almond oil; linseed oil.

INTRODUCTION: With a view to producing quality products, verifying compliance with food standards and labelling legislation, and to detect food fraud, European standards include regular laboratory control of raw materials, by-products and finished products. There are several destructive and non-destructive techniques for food authentication that have been the most successful, including: DNA-based speciation techniques, trace elements and stable isotope ratios analysis, ¹H, ²H, ¹³C nuclear magnetic resonance, etc. Interfaced gas chromatography/mass spectrometry system (GC/MS) combined with a modern data system presents a powerful analytical tool of high specificity and sensitivity, essential in many areas of food chemistry.

OBJECTIVES: The objectives of the presented research were focused on the development of a novel and rapid semi-qualitative approach, utilizing GC/MS, as a sophisticated analytical equipment, and contemporary data processing tool, to differentiate edible oil samples made from various oilseeds (sunflower, almond, and linseed). These

results should be applicable in quality assessment of commercial oil products.

METHOD / DESIGN: Investigated samples of oil were obtained from the Oil Department at the Faculty of Technology, Novi Sad, Serbia: 2 samples of sunflower oil (SFO-1, SFO-2), 1 sample of almond (AO) and 1 sample of linseed oil (LSO). 10 µL of each oil sample was dissolved in methylene chloride, derivatized using TMSH and analyzed on a GC/MS device (EI 70 eV, DB-5 MS (30 m × 0.25 mm × 25 µm), inj. temp. 250°C, helium 0.8 mL/min, temp. program: 50-



130°C, 30°C/min and 130-300°C at 10°C/min). 1 µL of each sample was injected with a split ratio of 1:50.

RESULTS: The eluting lipid components were identified using mass spectrometer, by comparing their fragmentation spectra with Wiley7 and NIST14 mass spectra libraries. Automatically integrated surface areas of detected fatty acids (hexadecanoic C16:0, 9,12-octadecadienoic (Z,Z)- C18:2 n-6, 9-octadecenoic (Z)- C18:1 n-9, octadecanoic C18:0, 11-eicosenoic C20:1 n-9, eicosanoic C20:0, docosanoic C22:0, and tetracosanoic acid C24:0) in the form of their numerical matrices, were inserted into PAST program, in order to perform exploratory analysis of a GC/MS data.

The obtained dendrogram shows strong separations between investigated samples of linseed, almond and sunflower oil. Two analyzed sunflower oil samples (SFO-1 and SFO-2) are grouped together, showing strong similarities (~100%).

The highest value of a cophenetic correlation coefficient (0.9773) was obtained using a *paired group* algorithm and *kulczynski* similarity measure, thus suggesting that the presented tree represents the dissimilarities among observations very faithfully.

CONCLUSIONS: Obtained results show that it is possible to distinguish edible oil samples of various oilseed species, according to the corresponding botanical origin, in a rapid way, on a GC/MS instrument with contemporary data analysis tools, successfully avoiding the application of analytical standards and accurate, time-consuming qualitative and quantitative determinations.

RHEOLOGICAL PROPERTIES OF WHEAT BASED DOUGH WITH ADDITION OF ALTERNATIVE CEREAL FLOURS

Aleksandra Torbica¹, **Jelena Tomic**¹

¹ *Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

**Corresponding author: jelena.tomic@fins.uns.ac.rs*

KEYWORDS: cereals; dough; rheology;

INTRODUCTION: From nutritional point of view it is important to include cereals other than wheat in human diet. This imposes the need of those raw materials processing parameters definition. The viscoelastic properties of wheat dough are the most important parameters in bread making process. Bread wheat dough exhibits the viscoelastic behavior primarily influenced by protein and starch components, and depends of technological quality of wheat flour. Mixing dough from wheat and different cereals flours leads to rheological alterations at different level and directions in comparison to the control wheat dough. Rheological behavior of dough from flour blends is also influenced by mixing conditions, flour hydration and dough consistency. Dynamic oscillatory measurements are suitable to examine the structure and properties of dough giving the information about its viscous and elastic characteristics.

OBJECTIVES: The objective of this study was to determine the influence of alternative cereal flours addition on viscoelastic properties of dough samples based on wheat flour of poor technological quality.

METHOD / DESIGN: The materials used in this study were dough samples made from blends of wheat flour of extremely poor quality with addition of durum, barley, oat, rye, triticale, millet and sorghum flours in ratio 70:30. Doughs were prepared in the 4-g Micro-DoughLab at high speed kneading dough mode (120 rpm) and constant water levels. The viscoelastic behavior of doughs were investigated by dynamic oscillatory test (frequency sweep from 0.1 to 10 Hz at constant stress of 10 Pa) using a Haake Mars (Thermo Scientific, Germany). All the measurements were done at 25 °C, using 60 mm parallel plates. The gap was adjusted to 2 mm and the edges were trimmed with a knife. The dough sample was left to rest between the plates for 5 min before testing, so that the residual stresses would relax.

RESULTS: The obtained curves showed the rheological behavior of dough blend samples. The control wheat sample had the lowest values of storage moduli (G'). In all the cases, the values of storage moduli (G') were higher than G'' , which showed that the doughs were more elastic than viscous. $\tan \delta$ (G''/G') values were in the range 0.3-0.4. These results indicated that applied cereal flours could be added to wheat flour without deterioration of processing parameters while some of them could be used as

wheat flour improvers. In order to obtain more reliable information, this experiment should be repeated applying the same consistency of dough samples.

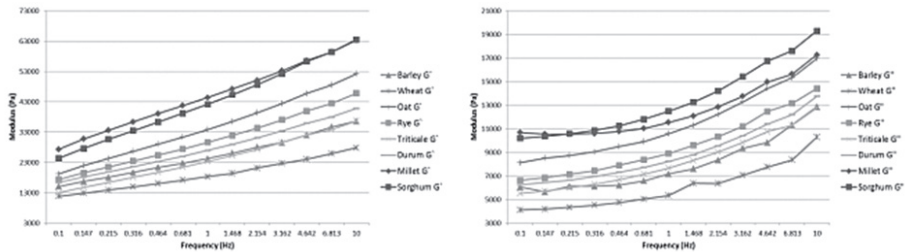


Figure 1. Storage modulus (G') and loss modulus (G'') for dough samples

CONCLUSIONS: The alternative cereals could be used not only for nutritive value added bakery products, but for the wheat flour technological quality improvers.

INFLUENCE OF TRITICALE AND RYE FLOUR ON TEXTURAL PROPERTIES OF WHEAT FLOUR BASED BREAD**Jelena Tomić¹**, Dragan Živančev¹, Aleksandra Torbica¹¹ *Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia***Corresponding author: jelena.tomic@fins.uns.ac.rs***KEYWORDS:** wheat; triticale; rye; bread

INTRODUCTION: It is well-known that wheat proteins are the most responsible for dough and textural properties of wheat bread gained by conventional breadmaking processes. Currently, one of the most important tasks in baking industry is to find raw material which will in combination with wheat flour gain bread of satisfying properties. Especially, the most demanding challenge is to estimate the influence of different raw materials in dependence on different wheat flour quality. Although rye has been used as raw material for bread production, rye proteins in dough are not capable to form such continuous protein network as wheat can. Its bread making performance is mainly based on the swelling properties of endogenous pentosans and because rye products are an important source of dietary fibre. Also, rye contains phenolic compounds, such as phenolic acids, alkylresorcinols and lignans. On the other hand, triticale is similar to wheat because it is created by crossing species of wheat and rye. Beside the same level of proteins as wheat, triticale possesses higher contents of lysine, methionine and threonine amino acids.

OBJECTIVES: The aim of this work is to examine the influence of triticale and rye flours addition on textural properties of bread based on wheat flour of poor technological quality.

METHOD / DESIGN: Wheat flour of extremely poor technological quality and rye flour were commercial samples whereas triticale flour (cultivar "Odisej") was obtained from Institute of Field and Vegetable Crops in Novi Sad. Standard procedure of breadmaking was applied for control (wheat) and flour blends (rye/triticale in ratio 10:90 of wheat flour). Protein content of flours is determined by Kjeldahl method. Empirical rheology test of wheat dough and these two mixtures was determined by Farinograph and Texture Profile Analyses (TPA) of 10 g of bread samples after 1 h of baking.

RESULTS: As can be expected, protein content of wheat flour was very low (6.55%). Although protein content of rye flour was higher compared to the triticale flour (12.90% and 11.22% respectively), addition of triticale flour positively influence some farinograph parameters. Namely, water absorption of dough with triticale flour was higher for 2.3 % than control wheat dough whereas degree of softening was lower for 20 BU and Quality number was at the same level like control sample. Addition of rye flour negatively influenced the Quality number, this value was lower for 9.9 than

for control wheat dough. According to literature data rye possesses higher pentosans content (12.20%) than wheat and triticale (6.60% and 7.60% respectively) and could be expected that bread with rye flour possess softer structure. However, the result of TPA showed that addition of triticale flour gave lower values of hardness and chewiness of bread in comparison to control wheat bread making it more sensory acceptable. Addition of rye did not have such impact. The reason for such behaviour of triticale flour may be the fact that triticale inherited protein quality from its wheat parent or that wheat and triticale proteins during dough mixing are making synergistic effect, but to prove these hypotheses it is necessary to carry out much more extensive surveys.

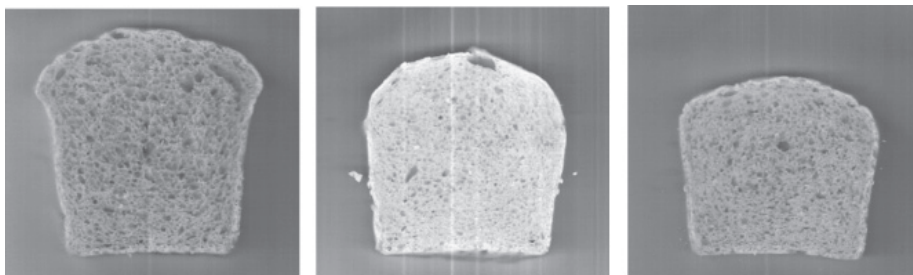


Figure 1. Cut loaves of bread produced from wheat flour with addition of 10% triticale flour, from pure wheat flour and wheat flour with addition of 10% rye flour

CONCLUSIONS: It could be concluded that addition of only 10% of triticale flour to wheat flour of poor technological quality improved texture properties of bread gained by standard procedure whereas addition of same percentage of rye flour to wheat flour did not show such effect. Also, this indicates that triticale flour is more convenient than rye flour for conventional breadmaking processes.

STEREOLOGICAL ANALYSIS OF RAT HEPATOCYTES AFTER SUBCHRONIC ACRYLAMIDE TREATMENT

Jelena Marković¹, Aleksandra Denić¹, Renata Kovač¹, Milica Matavulj¹

¹ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

*Corresponding author: JelenaMarković: jelena.markovic@dbe.uns.ac.rs

KEYWORDS: acrylamide; hepatocyte; stereology

INTRODUCTION: Acrylamide (AA) is carcinogen, mutagen and neurotoxic substance present in fried, roasted and baked starch-based goods. AA is formed in Maillard reaction from asparagine and carbonyl sources, such as reducing sugars, during thermal food processing at temperatures between 120 and 180°C.

OBJECTIVES: Since acrylamide is showing diverse harmful effects on variety of organ systems, the aim of our study was to inspect whether acrylamide exerts adverse effects on rat liver by light microscopic *stereological analysis*.

METHOD / DESIGN: The investigation was conducted on adult male Wistar rats aged 65 days at the beginning of the experiment. Thirty rats were divided into three groups, one control and two groups subchronically treated with 25 mg/kg bw and 50 mg/kg bw of AA respectively, during three weeks. Stereological analysis of rat hepatocytes was performed on 5 µm thick liver sections stained with *histochemical staining technique*. The following *stereology parameters* were determined: the volume density of hepatocytes (Vvh), volume density of hepatocyte nuclei (Vvhn), volume density of hepatocyte cytoplasm (Vvhc) and their nucleocytoplasmic (N/C) ratio.

RESULTS: Results of stereological analyses of hepatocytes are shown in Table 1.

Table 1. Examined stereological parameters of the hepatocytes of the control and the acrylamide-treated rats. The volume density of: hepatocytes (Vvh), hepatocyte cytoplasm (Vvh_c), hepatocyte nuclei (Vvh_n) and nucleocytoplasmic ratio (N/C). All results are expressed as the means±SEM.

Stereological parameter	Control	AA 25 mg/kg bw	AA 50 mg/kg bw
Vvh (mm ³ /mm ³)	0.993320±0.001524	0.993814±0.001620	0.997914±0.000740
Vvhc (mm ³ /mm ³)	0.843200±0.007664	0.836316±0.002529	0.797857*±0.004317
Vvhn (mm ³ /mm ³)	0.150100±0.006157	0.157498±0.001340	0.1915814*±0.004255
N/C ratio	0.178687±0.178687	0.188461±0.002094	0.237736*±0.006511

*Indicate significant differences (Kruskal–Wallis test, p<0.05)

CONCLUSIONS: Volume density of hepatocytes, hepatocyte nuclei and nucleocytoplasmic ratio showed dose-dependent increment in both AA-treated groups compared to the control, while volume density of hepatocyte cytoplasm was dose-dependently decreased in AA-treated groups compared to the control. Detected stereological parameters for hepatocyte showed statistically significant difference for all observed parameters, except for V_{vh} . Statistical analysis showed that V_{vh}_c , V_{vh}_n and N/C ratio were not statistically different between the control group and the group treated with acrylamide in dose of 25 mg/kg bw ($p>0.05$), but it revealed a statistically significant difference, for all three stereological parameters, between group treated with acrylamide in dose of 50 mg/kg bw and the control ($p<0.05$). Obtained results indicate that acrylamide, by changing hepatocyte microstructure, exerts a potentially adverse effect on the liver.

ACRYLAMIDE TREATMENT ALTERS LEVELS OF OXIDATIVE STRESS BIOMARKERS IN PANCREATIC BETA CELLS

Jelena Marković¹, Milena Stošić², Danijela Kojić¹, Snežana Orčić¹, Tatjana Nikolić¹, Milica Matavulj¹

¹ University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology

² University of Novi Sad, Faculty of Technical Sciences, Department of Environmental Engineering and Occupational Safety and Health

*Corresponding author: Jelena Marković: jelena.markovic@dbe.uns.ac.rs

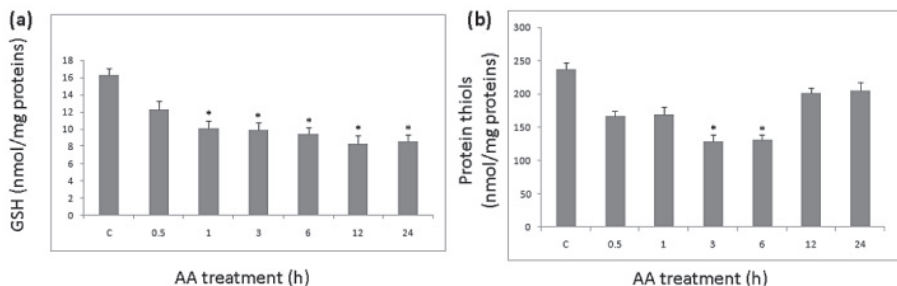
KEYWORDS: acrylamide; pancreatic beta cells; glutathione; protein thiols; lipid peroxidation

INTRODUCTION: Acrylamide is toxic chemical that can be formed as a food contaminant during the high-temperature cooking of many common foods such as potato products, breads and coffee. Although it is proven that acrylamide exposure induces oxidative stress in liver, testis, brain and kidney and causes endocrine disruption of thyroid gland, testis and mammary gland, acrylamide effects on pancreas, especially endocrine pancreas, are very scarce.

OBJECTIVES: The objective of our study was to determine whether acrylamide treatment disturbs redox balance by altering glutathione (GSH), protein thiols and malondialdehyde levels in rat pancreatic insulinoma cell line (Rin-5F).

METHOD / DESIGN: Rat pancreatic insulinoma cell line (Rin-5F) was treated with 10 mM acrylamide (IC_{50}) at time intervals of 0.5, 1, 3, 6, 12 and 24 h. After ultrasonic cell lysis in 2.5% sulfocalicylic acid, supernatant was analysed for the content of glutathione, while acid-precipitated proteins were used for protein thiols measurement. Lipid peroxidation was evaluated using thiobarbituric acid reactive substance assay (TBARS).

RESULTS: Detected GSH, protein thiols and malondialdehyde levels in rat pancreatic insulinoma cell line (Rin-5F) after acrylamide treatment are shown in Figure 1.



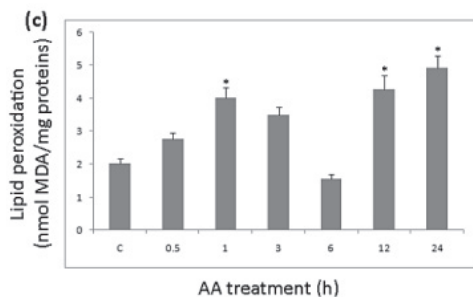


Figure 1. Assessment of redox status of Rin-5F cells after acrylamide (AA) treatment. Concentration of reduced glutathione (GSH) (a), protein thiols (b) and malondialdehyde (MDA) (c). All results are expressed as the means \pm SEM. * Mean values were significantly different from those of untreated control cells ($p < 0.05$).

CONCLUSIONS: In rat pancreatic insulinoma cell line (Rin-5F) the GSH level was dose-dependently decreased after acrylamide exposition. Except for 0.5 h long treatment, for treatments at all other time points there were *statistically significant differences* between AA treated and control samples. Although, acrylamide treatment reduced protein thiol content, only 3 h and 6 h long treatments showed significant decrease of thiols levels. Except for 6 h long treatment, acrylamide exposition led to increase in the lipid peroxidation level in comparison with control cells. However, only after 1 h, 12 h and 24 h long treatment, lipid peroxidation exhibited statistically significant induction. Obtained data implies that acrylamide treatment alters levels of oxidative stress biomarkers in pancreatic beta cells. Disturbed redox balance could cause beta cell dysfunction and impaired the insulin secretion.

FATTY ACID PROFILES OF POLLEN COLLECTED FROM SIX SERBIAN MAIZE HYBRIDS

Aleksandar Ž. Kostić¹, **Ivana I. Jevtić**², Bojana D. Špirović-Trifunović³, Jelena B. Popović-Đorđević¹, Mirjana B. Pešić¹, Marina P. Mačukanović-Jocić⁴

¹ Faculty of Agriculture, Chair of Chemistry and Biochemistry, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

² Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia

³ Faculty of Agriculture, Chair of Pesticides, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

⁴ Faculty of Agriculture, Chair of Agrobotany, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

*Corresponding author: akostic@agrif.bg.ac.rs

KEYWORDS: maize; pollen; fatty acids

INTRODUCTION: Lipid substances in the pollen have different important roles – nutritional, protective or attractive for pollinators. Depending on the type, lipid substances can be found in all parts of pollen grain but the most important is surface thin layer known as pollen kitt zone. Fatty acids (FA) are an important part of lipid fraction in pollen. They can be present either in free form or as esters with some alcohols.

OBJECTIVES: The aim of this work was to identify and quantify fatty acids present in maize pollen samples collected during June 2015: from six different hybrids developed at the Maize Research Institute (Zemun Polje). This Institute represents the leading scientific institution for maize research in Serbia and Balkans.

METHOD / DESIGN: After collection all samples were packed in vacuum bags and kept in freeze until further analysis. Lipid fraction was extracted from pollen using ultrasonic bath for 15 min with heptane as solvent under strong agitation. Subsequently, the solvent was evaporated to dryness by rotary evaporation. After that content of fatty acids (%) was determined by GC analysis.

RESULTS: According to results of GC analysis, in total, twenty eight fatty acids are identified in pollen samples. Sample 3 (ZP 611K) contains twenty FA, sample 6 (ZP 5557 Lady Fingers) contains fourteen FA, samples 1 (ZP Standard yellow grain), 2 (ZP 608K) and 5 (ZP Rumenko) contain thirteen FA and sample 4 (Sweet corn L620/121) contains only eleven FA. Two fatty acids are found in all samples: C18:0 and C18:2n6-c. Five samples contain C15:0, C16:0, C18:2n6-t and C21:0 FA, four samples contain C12:0, C14:0, C18:3n-3 and C20:2 FA while three samples contain C6:0, C10:0, C11:0; C18:1n9-c and C18:3n-6 FA. The rest of the FA were found in two (C13:0; C15:1; C16:1; C17:0; C17:1; C18:1n-9t; C20:3n-6; C22:2) or only in one (C8:0; C20:4n-6; C23:0; C20:5n-3; C24:0) of the investigated samples so they could be characterized as “rare fatty acids”. The most abundant saturated FA was palmitic acid (16:0) in four samples (3, 4, 5 and 6) and henicosoanoic acid (21:0) in samples 1 and 2. Most

prevalent monounsaturated FAs were oleic acid (18:1n9c) in three samples (2, 3 and 4), elaidic acid (18:1n9t) in two samples (1 and 6) and *cis*-10-heptadecenoic acid in sample 5. Linoleic acid (18:2n6-c) was the most abundant polyunsaturated fatty acid in five samples (1, 2, 4, 5 and 6) while *cis*-11,14-eicosadienoic acid (20:2) was with maximum amount in sample 3 and equal with linoleic acid in sample 1.

CONCLUSIONS: This research has shown a great variety in the composition and quantity of fatty acids present. A significant presence of unsaturated fatty acid that is important for the nutritional value of pollen may also be observed. The presence of fatty acids with odd C-number carbon chains can be noticed in all samples which is not such a common phenomenon.

The research was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant Nos. TR 31069 and 46009), as well as by EU Commission Project AREA, No. 316004.

HEDONIC PERCEPTIONS OF FUNCTIONAL PASTA: COMPARISON BETWEEN REGULAR AND IRREGULAR CONSUMERS OF FUNCTIONAL PASTA

Dubravka Škrobot¹, Mladenka Pestorić¹, Alena Tomšik¹, Miona Belović¹, Ivan Milovanović¹, Bojana Šarić¹, Anamarija Mandić¹

¹ University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*Corresponding author: dubravka.jambrec@fins.uns.ac.rs

KEYWORDS: pasta, buckwheat, sensory evaluation, hedonic perception, consumer

INTRODUCTION: Consumers are increasingly becoming aware of health benefits of food. Accompanying new trends in eating habits and consumers demand towards the healthier foods, manufacturers together with researchers have been looking for the ingredients that can be added into the basic food formulation in order to improve its nutritional and functional benefits. However, formulation changes could cause dissatisfaction in consumers with the sensory quality, due to changes in colour, texture, taste or odour.

OBJECTIVES: Conducting sensory evaluation only to consumers which are not regular consumers of special types of pasta may have little significance. The purpose of this paper was therefore to compare hedonic perception of regular (consumers that have been eating special types of pasta several times; RC) and irregular (consumers that have not eaten special types of pasta; NRC) consumers of special types of pasta.

METHOD / DESIGN: All subjects evaluated overall liking, colour acceptability, texture acceptability and taste acceptability of pasta samples. Hedonic data were submitted to two-way ANOVA considering samples, subjects and their interaction as factors.

RESULTS: Results showed that the main factor Subject ($F_{1, 266}=2.907$, $p=0.089$) was not significant only for pasta texture acceptability of samples, indicating that both consumer groups perceive pasta texture in a similar manner. Furthermore, results showed that the main factor Sample ($F_{1, 266}=1.089$, $p=0.369$) was not significant only for pasta colour acceptability, suggesting that previous experience in eating functional pasta does not influence colour assessment.

In general, there is agreement between the two groups of consumers in the case of ranking pasta samples for overall liking, however, regular consumers scored higher evaluated sensory properties of pasta samples in comparison to non-regular consumers.

CONCLUSIONS: The results obtained in this study indicate that there are increases in acceptability due to habitual consumption of special types of pasta.

ASSOCIATION MAPPING FOR AGRONOMIC TRAITS IN NS WHEAT CORE COLLECTION

Dragana Trkulja¹, Ljiljana Brbaklić², Ankica Kondić-Špika¹, Borislav Kobiljski², Sanja Mikić¹, Marina Čeran¹, Svetlana Glogovac¹

¹ *Institute of Field and Vegetable Crops, Serbia*, ² *Biogramum, Serbia*

*Corresponding author: dragana.trkulja@nsseme.com

KEYWORDS: association mapping; microsatellites; QTL; wheat; yield

INTRODUCTION: Association mapping presents a perspective method for genetic dissection of complex traits. Here, detection of number and location of genes for some agronomically important trait relies on occurring variation in diverse germplasm and on historical recombination events within wheat. Association analysis is based on linkage disequilibrium concept, defined as the non-random co-segregation of alleles at two loci. Comparing to traditional quantitative trait mapping, where mapping populations are used, association mapping gives maps with better resolution. Apart from that, this method is less time consuming since no mapping populations need to be generated.

OBJECTIVES: The aim of this paper was to detect stable QTLs (Quantitative Trait Loci) associated with four agronomically important traits in a wheat core collection of the Institute of Field and Vegetable Crops, Novi Sad, Serbia.

METHOD / DESIGN: A set of 31 microsatellite loci, positioned along almost all three genomes and located near previously detected important QTLs, was used for molecular evaluation. The material consists 282 wheat accessions originated from 26 countries worldwide, contrasting in expression for yield related traits. Genomic DNA from all genotypes was extracted from young leaves according to the CTAB protocol (Doyle & Doyle, 1990). The PCR products were analyzed using the capillary electrophoresis on an ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The population structure was obtained by the Structure software (Pritchard et al., 2000) and additionally validated through principal coordinate analysis (PCoA) employing Excel add-in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Phenotypic evaluation of four traits (heading time, flowering time, stem height and spike length) was performed at Rimski Šančevi, Novi Sad, Serbia, during ten growing seasons, from 2000 to 2009. The presence of marker-trait associations was assessed using the mixed linear model (MLM) in Tassel software (Bradbury et al., 2007).

RESULTS: The population structure revealed existence of 3 subpopulations and grouping was in accordance with geographic origin and pedigree of analyzed accessions. Association analysis indicated presence of 103 marker-trait associations, with highest number for flowering time (32) and lowest for stem height (20). Five markers were associated with more than one trait in at least 3 out of 10 analyzed years (Table 1).

T5

Table 1. Marker-trait associations detected ($p \leq 0.05$) in more than three evaluated years using the MLM

Trait / Marker	BARC212-2A	GWM425-2A	WMC177-2A	GWM261-2D	BARC164-3B
Heading time	7	9	ns	7	6
Flowering time	8	4	ns	7	8
Stem height	7	ns	3	ns	ns
Spike length	ns	5	6	6	ns

CONCLUSIONS: Obtained results showed presence of stable QTLs for analyzed traits, on chromosomes 2A, 2D and 3B and associated markers can be used for marker assisted selection in Serbian agro-ecological conditions.

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INVESTIGATION OF ANTIOXIDANT STABILITY OF WILD APPLE FRUIT EXTRACTS

Dragana Stojiljković¹, Ivana Arsić², Sonja Roganović², Vanja Tadić³

¹ Health Care Institution Pharmacy "Farmakop", Niš, Serbia,

² Department of Pharmacy, Faculty of Medicine, University of Niš, Serbia,

³ Department for Pharmaceutical Research and Development, Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia

*Corresponding author: s.dragana83@yahoo.com

KEYWORDS: wild apple fruit extracts; bioactive polyphenolic compounds; antioxidant activity

INTRODUCTION: The use of plant extracts rich in natural antioxidant compounds, primarily in polyphenolic compounds, is current for many years, and is very important for strengthening of protective endogenous system, as well as in the prevention and/or treatment of damages and diseases caused by oxidative stress. These bioactive natural antioxidants can reduce ultraviolet rays penetration, protect body from oxidative stress, prevent reactive oxygen species formation, cell photoaging and development of many diseases. Interest in the application of natural antioxidant substances is steadily increasing primarily because of their safe use, too. Wild apple fruit (*Malus sylvestris* (L.) Mill., Rosaceae) contains a large number of biologically active antioxidant substances, including polyphenolic compounds, and because of that its extracts can be potentially used in protection and prevention of many oxidative stress-related diseases (cardiovascular or degenerative diseases, atherosclerosis, diabetes, osteoporosis, cancer, dermatitis, phototoxicity). Investigated wild apple represents a biomarker of the territory of Serbia, and a plant used in Serbian folk medicine.

OBJECTIVES: The aim of this research was to determine the polyphenolic content and antioxidant activity of ethanol extracts of wild apple fruit (EEWAF), originated from southern Serbia, obtained by different extraction methods in order to investigate possibility of their use as a source of bioactive substances with antioxidant effects in food industry or dietary supplements productions.

METHOD / DESIGN: Extracts of wild apple fruit were prepared with 70% ethanol as a solvent by maceration (EEWAF-M) and ultrasonic extraction (EEWAF-U), in drug:extract ratio 1:5. Total content of phenols (TPC) was determined by Folin-Ciocalteu test and expressed as gallic acid equivalents (GAE), flavonoid (TFC) by Markham's method, with rutin as standard (RE), tannins (TTC) with vanillin test and expressed as catechin equivalent (CE), while the percentage content of total anthocyanins (TAC) was determined by Eur.Ph.6.0. Antioxidant activity (AA) was determined by DPPH (1,1-diphenyl-2-5-picryl-hydrazyl) test and expressed as %RSC (Radical Scavenging Capacity). AA was measured immediately after preparation and after six months of storage at room temperature.

T5

RESULTS: All EEWAF showed a good content of polyphenolic compounds and good antioxidant activity during the entire study period. The evidence of correlation between AA of extracts and polyphenolic compounds content has been shown to be good. Antioxidant activity, generally, increased proportionally with increasing content of polyphenolic compounds. Type of extraction method used had important influence on content of polyphenolic compounds and AA. EEWAF-U showed a better content of bioactive polyphenolic substances compared to EEWAF-M, as well as AA. Namely, TPC in EEWAF-M and EEWAF-U were 960.58 and 1223.65 mgGAE/100g dry weight (dw), TFC were 57.55 and 64.91 mgRE/100g dw, TTC were 154.55 and 181.82 mgCE/100g dw, and TAC were 1.95 and 2.93 %, respectively. Better ability to neutralize free radicals showed EEWAF-U and it showed better %RSC (78.43 %RSC) compared to EEWAF-M (70.96 %RSC). After six months of storage, extracts maintain their good AA. %RSC was lower, but still satisfying. EEWAF-U showed better %RSC also after six months (58.96 %RSC) compared to EEWAF-M (50.46 %RSC).

CONCLUSIONS: Interesting results were obtained for the investigated EEWAF proving to be rich in bioactive polyphenolic compounds and demonstrating a good AA, immediately after preparation and after six months, while giving the evidence of good correlation between antioxidant properties and phenolic compounds content, as well. The results of our study indicated that wild apple fruit might be taken into consideration as a source of bioactive natural antioxidant substances for potential use in food industry.

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FUNGAL CONTAMINATION OF GRAIN AND FLOUR OF WHEAT, CORN AND BUCKWHEAT

Dragana Plavšić¹, Marija Škrinjar², Đorđe Psodorov¹, Ljubiša Šarić¹,
Dragan Psodorov³, Ana Varga¹, Anamarija Mandić¹

¹ *Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

² *Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*

³ *College of Management and Business Communications, 21205 Sremski Karlovci, Mitropolita Stratimirovića 110, Serbia*

*Corresponding author: dragana.plavsic@fins.uns.ac.rs

KEYWORDS: wheat, corn, buckwheat, flour, molds.

According to the nutritive characteristics, the wholegrain flour is a high quality product, due to its high vitamin, mineral, and dietary fibers content. However, the cereal grains are susceptible to the series of contamination during the ripening, harvesting, processing and storage.

The aim of this work was to determine molds presence in grain and flour of wheat, corn and buckwheat. The total number and determination of isolated genera and species of molds were subject of this research.

All samples were contaminated by the molds. Eight fungal genera (*Alternaria*, *Aspergillus*, *Cladosporium*, *Chrysonilia*, *Fusarium*, *Penicillium*, *Rhizopus* and *Scopulariopsis*) and fifteen species were isolated. The highest number of mold species was isolated from the genus *Aspergillus*. About 66.7% isolated fungi belonged to potentially toxic species.

The results are pointing out a necessity of the grain surface treatment, preceding the milling in wheat, corn and wholegrain buckwheat flour production.

SUNFLOWER BREEDING FOR RESISTANCE TO BROOMRAPE – AN INTEGRATED APPROACH

Dragana Miladinović¹, Siniša Jocić¹, Sandra Cvejić¹, Ivana Imerovski¹, Aleksandra Dimitrijević¹, Boško Dedić¹, Miguel Cantamutto²

¹ *Institute of Field and Vegetable Crops, Novi Sad, Serbia*

² *INTA, Hilario Ascasubi, Argentina*

*Corresponding author: dragana.miladinovic@ifvcns.ns.ac.rs

KEYWORDS: sunflower; broomrape; resistance

INTRODUCTION: Broomrape (*Orobanche cumana* Wallr.) is by far the most economically damaging parasitic weed in sunflower and is regarded as the most important constraint in sunflower production in European countries, as well as in the Middle East and Asia. Currently, *O. cumana* is causing worldwide yield losses between 5-100%. The lack of coordinated interdisciplinary involvement has been a major constraint that has impeded progress in the sustainable control of broomrape in sunflower.

OBJECTIVES: The aim of the research was to integrate research on both broomrape and sunflower focusing on environmental, pathogenic, physiological, and molecular genetic factors affecting the sunflower-broomrape interaction and sunflower resistance to this parasite.

METHOD / DESIGN: Analysis of soil and climate conditions in broomrape affected and non-affected regions was done in order to evaluate the effect of abiotic factors on broomrape occurrence. At the same time, molecular characterization of broomrape ecotypes was done, for estimation of the intra- and inter-ecotype variability. In order to identify potential new sources of resistance, sunflower germplasm was screened for broomrape resistance in field and greenhouse conditions.

RESULTS: Some of the observed climate and soil parameters had an effect on broomrape occurrence to a certain extent. The P availability in non-invaded habitats was higher than in broomrape invaded habitats, indicating that phosphorous fertilization could to be a candidate technology to limit the broomrape damage. Molecular analysis showed great variability of broomrape ecotypes, both from Serbia and abroad. Field and greenhouse tests revealed genotypes with either resistance or tolerance to new, virulent broomrape races.

CONCLUSIONS: The integrated research on both broomrape and sunflower focusing on environmental, pathogenic, physiological, and molecular genetic factors affecting the sunflower-broomrape interaction and sunflower resistance to this parasite has proved to be useful tool for making progress in the sustainable control of broomrape in sunflower, thus contributing to more safe and stable sunflower production.

CHEMICAL PROFILE OF SELECTED MEDICINAL PLANTS (PEPPERMINT, BASIL AND MARIGOLD) FROM CONVENTIONAL AND ORGANIC PRODUCTION

Branimir Pavlič¹, Jelena Vladić¹, Aleksandra Gavarić¹, Dušan Adamović², Senka Vidović¹, Zoran Zeković^{1,*}

¹ Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia,

² Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

*Corresponding author: zzekovic@tf.uns.ac.rs

KEYWORDS: Medicinal plants; organic production; extraction; chemical profile; bioactive compounds

INTRODUCTION: Nowadays, investigation of medicinal and aromatic plants is gaining more and more attention, with the aim to obtain extracts which could be potentially applied in pharmaceutical, cosmetic or food industry. Also, the most commonly expressed motives for purchasing organic food have become consideration for the environment and certain health reasons.

OBJECTIVES: The main objective of this work was comparison of conventionally and organic grown medicinal plants: peppermint (*Mentha piperita*), basil (*Ocimum basilicum*) and marigold (*Calendula officinalis*). Various extraction techniques (both traditional and novel) were applied in order to isolate target bioactive compounds from investigated plants.

METHOD / DESIGN: Hydrodistillation, Soxhlet extraction with organic solvent and supercritical fluid extraction were applied, in order to extract essential oil and lipid compounds. Essential oils and lipid extracts were fingerprinted for volatile (terpenoid) compounds by HPTLC. On the other hand, maceration and ultrasound-assisted extraction were used for isolation of polyphenolic fraction, while total phenolic content, total flavonoid content and antioxidant activity were determined in obtained liquid extracts.

RESULTS:

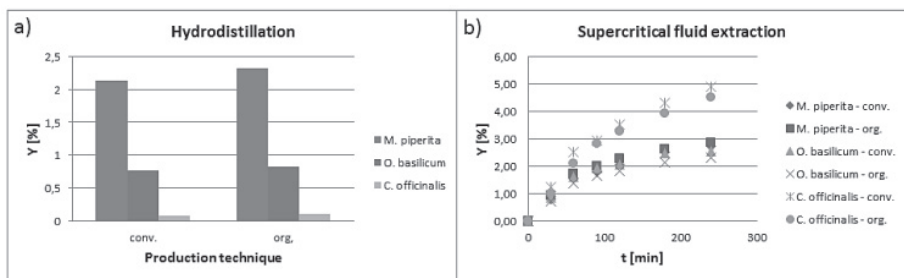


Figure 1. Yield of essential oil (a) and lipid extract (b) obtained by hydrodistillation and supercritical fluid extraction

CONCLUSIONS: According to results, it could be concluded that organic production of medicinal plants could be beneficial in terms of yield and content of certain bioactive compounds (essential oil and polyphenolic fraction). However, in case of lipid extracts, there was no significant difference in extraction yield obtained by Soxhlet and supercritical fluid extraction from conventionally and organic grown medicinal plants.

BIO-REFINING OF FILTER TEA FACTORY BY-PRODUCTS: CLASSICAL AND ULTRASOUND ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM WILD APPLE DUST

Abdulkhkim Naffati¹, Jelena Vladić¹, **Branimir Pavlič¹**,
Milan Blagojević², Senka Vidović^{1,*}

¹University of Novi Sad, Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia

²University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

*Corresponding author: senka.curcin@yahoo.com

KEYWORDS: wild apple; by-product; phenolic compounds; ultrasound assisted extraction; optimization

INTRODUCTION: Filter tea industry generates a wide range of different herbal and fruit dusts, as it processes many different kinds of plant materials. Quantity of some of them is significant and varies from region to region. Target of this research was fruit dust left after production of filter tea that contains wild apple. As several studies have shown apples have many health benefits, among most important are the reduction of the risk of cardiovascular diseases and cholesterol. In apple extracts number of different phenolic compounds can be expected: chlorogenic acid, quercetin, isorhamnetin, epicatechin, procyanidin dimer A etc. During the wild apple processing in filter tea factory app. 20% of wild apple fruit dust is produced calculated on input raw material. Having in mind quantity of this by-product and quality of wild apple (biological activity and chemical composition) it is easy to target the wild apple fruit dust as by-product worth to be “recycled”. One of the best ways to utilize this kind of material is through application of solid-liquid extraction (SLE).

OBJECTIVES: The main objective of this study was to explore the possibility of SLE to be applied as technique for wild apple fruit dust utilization. Besides, one of the research main aims was to optimize the process conditions, for more efficient SLE technique, for the production of wild apple liquid extract of desirable characteristics (high concentration of antioxidant compounds). Evaluation of wild apple herbal dust quality in comparison to other apple by-products has been provided to.

METHOD / DESIGN: In this study classical extraction (CE) and ultrasound-assisted extraction (UAE) have been applied for the extraction of bioactive compounds from wild apple fruit dust. In CE water and following mixtures of water and ethanol were used: 30%, 50% 70% and 90% ethanol. 17 experiments were set in the experiment on ultrasound assisted extraction where 70% ethanol was used as extraction solvent. In order to achieve the highest possible extraction of targeted compounds, optimization, by application of Response Surface Methodology, for more efficient extraction technique has been done. Influence of several process parameters was observed: extraction solvent, ultrasonic power, temperature and time. Observed responses were:

total phenolic (TP) and flavonoid content (TF), antioxidant activity (IC_{50}) and extract reducing power (EC_{50}).

RESULTS: According to the results content of TP in wild apple fruit dust ranged from 777.003 to 1148.030 mg GAE/100 g of investigated material. In CE among ethanol/water mixtures, highest efficiency for TP and TF extraction from wild apple dust showed 50% ethanol content of totally extracted phenols was 29.52 mg GAE/g and flavonoids 18.34 mg KE/g. However, in the case of extraction by 50% ethanol, probably due to increased extraction of concomitant compounds, efficiency of the following filtration process was difficult and unsatisfactory. According to the results on UAE of wild apple fruit dust the highest extraction yield of TP (57.49 mg GAE/g) was obtained by the application of ultrasound of 144 W for 60 minutes, while temperature was set at 60 °C. The lowest yield (25.55 mg GAE/g) was obtained by setting extraction parameters at 40 °C for temperature, 40 min for extraction time, and 144 W for ultrasonic power. The most dominant and highly significant factor that affects the extraction of TP from the wild apple dust is temperature and time interaction. The highest antioxidant activity was obtained in extracts prepared at the temperature of 80 °C, for 60 min with the application of ultrasonic power of 72 W. Linear terms of all three parameters investigated in UAE of wild apple dust effect antioxidant activity of obtained extracts. The effect of linear term of time is moderate, while effects of linear terms of temperature (negative) and ultrasound power (positive) are significant.

CONCLUSIONS: The results of this study show that the food industry by-products and waste, such as wild apple fruit dust (by-product of filter tea industry) can be utilized and recycled into highly valuable products, such as liquid extracts, thus making the whole production more efficient. The results of the study also show that both methods of extraction, classical and UAE by ethanol-water mixtures can be applied for the production of quality extracts. Obtained extracts were of good quality in terms of high concentration of phenolic compounds and high antioxidant activity. However, UAE was much more efficient method which yielded extracts of much higher concentration of targeted compounds, TP and TF.

INFLUENCE OF PRIMING ON GERMINATION PARAMETERS OF SOYBEAN SEED

Zlatica Miladinov¹, Svetlana Balešević-Tubić¹, Vojin Đukić¹, Jelica Veselić¹

¹ *Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia*

*Corresponding author: zlatica.miladinov@ifvcns.ns.ac.rs

KEYWORDS: germination, germination parameters, priming, soybean seed

INTRODUCTION: Seed priming is a process which leads to fast and even germination and sprouting with the aim of achieving high vigour. This process has practical agronomic significance, especially in unfavorable environmental conditions. Priming ensures optimum running of molecular-biological processes during germination, stimulates activation of different enzymes, mobilizes proteins reserves and prepares cells for division.

OBJECTIVES: The aim of the study was to examine the effect of primers on seed quality parameters: germination (GE) mean germination time (MGT), germination index (GI), and time to 50% germination (T50), under different values for initial seed germination.

METHOD / DESIGN: Seeds of ten selected soybean lines (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10) were used in the study, while the lines were grown within the breeding program of Institute of Field and Vegetable Crops Novi Sad. Initial seed germination values ranged from 48% to 89%. The seed was surface-sterilized with a 3% solution of sodium hypochlorite, and then immersed in different primers: potassium nitrate 1% (KNO₃), potassium chloride 1% (KCl), hydrogen peroxide 1% (H₂O₂), and untreated seed was used as control (C).

RESULTS: The findings showed that the effect of priming depends on the line and treatment, and that initial seed germination value has no effect on the value of pre-sowing treatment. Some lines had a favourable reaction to immersion, while inhibitory activity which occurred in other lines significantly decreased the values of seed germination parameters. Increase in germination ranged from 9-12% depending on the line and treatment, while decrease was found at 11%. Lines in which a positive effect was observed after applying this treatment, also had higher values of other germination parameters: mean germination time (MGT), germination index (GI), and time to 50% germination (T50).

CONCLUSIONS: On the whole, there is no universal application of only one primer, as it might not be suitable for a particular line and result in seed quality decrease.

SYNTHESIS OF THE MECHANISM FOR CONFIGURATION CHANGE OF THE SEEDBED CULTIVATOR

Ivan Knežević¹, Maja Čavić¹, **Miodrag Zlokolica**¹, Marko Penčić¹, Milan Rackov¹, SavoBojić¹, Jovica Surčinski¹

¹ Faculty of Technical Sciences, Novi Sad, Serbia

*Corresponding author: ivanknezevic@uns.ac.rs

KEYWORDS: seedbed cultivator; mechanism; transport position

INTRODUCTION: When designing agricultural machinery where a working width is larger than the dimensions that exceed the legally stipulated width of the vehicle during transport, there is a problem of changing configuration of machine from transport position to working position, and vice versa. If the assembly for the changing configuration from working to transport position is realized (designed) only with hydraulic cylinder, then the machine behaves as a rigid system that does not have the ability to adapt to the land, which is acceptable only for small working widths.

OBJECTIVES: To solve this problem it is necessary to form an assembly for changing configuration of machine, which will consist of hydraulic components and mechanism mounted on machine frame. Also, for machines with larger working width (more than 6 meters), a solution for terrain tracking will be proposed.

METHOD / DESIGN: After detailed kinematical and dynamical analysis of the proposed mechanical system an optimization synthesis will be performed with emphasis on the important design parameters such as: overall dimensions, loads, cylinder parameters etc. Solution will be verified through virtual prototype simulation.

RESULTS: A new hydro-mechanical system for changing the configuration of seedbed cultivator during the transition from working to transport position is designed. The mechanism with the hydraulic cylinder is extended with two points with the addition of elastic element – spring, which allows customization of seedbed cultivator to the land conditions.

CONCLUSIONS: The assembly is robust, reliable, compact and applicable to many different machines without the need for major changes to the design.

INFLUENCE OF DIFFERENT CONCENTRATIONS OF GLYCEROL AND GUAR-XANTHAN ON PROPERTIES OF BIOPOLYMER COMPOSITE FILM

Sandra Bulut¹, Vera Lazić¹, Senka Popović¹, Nevena Hromiš¹, Danijela Šuput¹

¹ Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia

*Corresponding author: sandra.bulut@gmail.com

KEYWORDS: biopolymers; pumpkin oil cake; additives; composite films; properties

INTRODUCTION: The ability to decompose in environment, as well as fact that they are isolated from natural sources, make biopolymers one of the most interesting topics for researchers. Today, biopolymers have wide application in pharmacy, medicine, agro and food industry. In food industry, among other applications, their use is especially interesting as packaging materials for different products. They show good film forming ability and, as formed films, have good mechanical and barrier properties. However, without addition of plasticizers, stabilizers and other additives, obtained biopolymer-based films are, usually, very rigid and not handable. Meanwhile, these additives can have unwanted influence on properties of films, especially on barrier characteristics. That is why it is very important to examine appropriate plasticizers and stabilizers for their minimum concentration for obtaining films with best performance for packaging different food products with specific requirements.

OBJECTIVES: The objective of this study was to examine influence of glycerol and guar-xanthan in different concentrations on whole pumpkin oil cake (PuOC) film properties, and find film with best mechanical and barrier characteristics. In this paper different concentrations of glycerol (30, 40 and 50%) as the most commonly used plasticizer, and guar-xanthan (0.1, 0.3 and 0.5%) as a stabilizer, were added in PuOC layer of double-layer composite films based on PuOC and zein.

METHOD / DESIGN: The film-forming solution of PuOC (10%, w/w) in deionized water was produced with addition of glycerol and guar-xanthan in different concentrations. After adjusting pH = 12 and incubating at 90 °C, solutions were casted onto Teflon-coated Petri dishes and films were dried for 2 days. The film-forming solution of zein (10% w/v) was prepared by suspending zein in 85% ethanol with the addition of PEG 400 (50%), and incubating solution at 80 °C. Zein solution was casted on dried PuOC films. Film thickness was measured with micrometer with sensitivity of 0.001 mm. Tensile strength and elongation at break of films were measured on Instron Universal Testing Instrument Model No 4301 (Instron Engineering Corp., Canton, MA) according to the *ASTM standard method D882-01*. Total soluble matter, moisture content and swelling of films were determined according to *Hromiš et al., (2015)*. The water vapour permeability (WVP) was determined by the gravimetric method according to the standard ISO 2528:1995 (E) (the used test conditions: temperature 25

° C +/- 1 ° C; relative humidity 90% +/- 2%). Determination of the gas permeability (CO₂, N₂, O₂) was conducted in accordance with DIN 53380, with the use of the Lyssy GPM-200 apparatus with the belonging Gasukuro Kogyo GC-320 gas chromatograph and the HP 3396A integrator.

RESULTS: Tested films had thickness from 0.26 to 0.33 mm. Slight increase of film thickness was observed with increase of glycerol concentrations, for the same concentrations of guar-xanthan. The best mechanical properties were determined for the film with 30% of glycerol and 0.5% of guar-xanthan. Obtained results showed that higher concentrations of glycerol (above 30%) lowered tensile strength end elongation to break and, in this way, weakened films. Maximal tested concentration of guar-xanthan (0.5%) gave films with highest tensile strength as well as elongation at break. An increase of concentrations of guar-xanthan and glycerol, had slight influence on total soluble matter and degree of swelling of films, while influence on film solubility wasn't detected. Also, slight influence of higher concentrations of added film additives on increase of WVP of tested films was observed. However, the results showed that obtained composite films have very good barrier properties to O₂ (lower than 50 ml/m²/day at 1 bar), regardless of concentrations of glycerol and guar-xanthan. Meanwhile, an increase of CO₂ permeability was observed with an increase of glycerol and guar-xanthan concentration.

CONCLUSIONS: Obtained results showed that the best mechanical properties had film with the lowest concentration of glycerol (30%) and the highest concentration of guar-xanthan (0.5%). Obtained values for water vapour permeability are typical for biopolymer-based films. Sensitivity to moisture, due to their hydrophilic nature, limit their use for product with high percentage of water. However, all tested films showed very good barrier against O₂, which is one of the main requirements for food packing materials especially for very perishable products. With an increase of concentration of added substances, permeability of CO₂ also increased. Presence of CO₂ in package may have an important role for maintaining the quality and extending the shelf-life of foods. However, for products, for which process of respiration is characteristic, such as cheese, fresh fruits and vegetables, materials with higher permeability of CO₂ can be advantage.

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RECYCLING OF FOOD FACTORY BY-PRODUCTS: SUPERCRITICAL EXTRACTION OF YARROW HERBAL DUST FROM FILTER TEA FACTORY

Ana Vasić Đurković¹, Aleksandra Gavarić¹, Jelena Vladić¹, Stela Jokić², Krunoslav Aladić³, Senka Vidović¹

¹ University of Novi Sad Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia

² Josip Juraj Strossmayer University Osijek, Faculty of Food Technology, Franje Kuhača 20, 31000 Osijek Croatia

³ Croatian Veterinary Institute, Veterinary Department Vinkovci, 32100 Vinkovci, Croatia

*Corresponding author: senka.curcin@yahoo.com

KEYWORDS: supercritical extraction, by-product, herbal dust, yarrow, camphor, eucaliptol

INTRODUCTION: During the production of filter tea a certain amount of herbal material of particle size lower than the size of pores of filter bag material is produced. This kind of material, usually called „herbal or fruit dust“, is discharged from the production as by-product or waste. Among aromatic herbs in Serbia yarrow is one of the mostly used in such form of production. During yarrow processing approximately 15% of raw material is converted in to “herbal dust”. Yarrow is medicinal plant widely used in traditional medicine for various forms of disorders. The main active compounds of yarrow are flavonoids, tannins, coumarins, sterols, salicylic acid, isovaleric acid and asparagine, but the most important compound in yarrow is essential oil and containing aromatic compounds. Having in mind main bioactive compounds and nature of this kind of material the application of supercritical extraction by carbon dioxide could be one of the possible choices for yarrow herbal dust recycling and utilization. Supercritical extraction by carbon dioxide represent the green environmentally friendly technology. In comparison to others extraction it enable production of “ready to use” solvent free extracts.

OBJECTIVES: The main objective of this study was to apply supercritical carbon dioxide for production of low polar yarrow extracts with high concentration of aromatic bioactive compounds and no trace of extraction solvent. During investigation influence of main process parameters on extraction yield and extracts composition was analysed.

METHOD / DESIGN: Supercritical carbon dioxide extraction of yarrow herbal dust was performed at two different temperatures (40 and 60°C) and three different pressures (100, 200 and 300 bar). Extraction yield was measured after 30 min, 1, 1.5, 2, 3 and 5 hours, and using these results extraction kinetics was analysed. By application of GC/MS and GC FID qualitative profile and concentration of main aromatic compounds in obtained supercritical extracts was analysed.

RESULTS: Total extraction yield was in the rage from 0.769 to 6.003%, and it was increased with increase of process pressure. The highest extraction yield was obtained

by application of supercritical carbon dioxide at pressure of 300 bar and temperature of 40 °C, while lowest was observed at pressure of 100bar and temperature of 60 °C. In obtained extracts several aromatic constituents has been detected, among most important were: p-cimen, linalool, 4-terpineol, trans caryophyllene, eucalyptol, camphor, borneol, and gama and alpha terpinene. Dominant compounds in all extracts were eucalyptol and camphor. Highest concentration of both compounds (1.56 for eucalyptol and 3.52 g/100g of extracts for camphor) has been obtained in extract prepared using supercritical carbon dioxide at pressure of 200 bar and temperature of 60 °C.

CONCLUSIONS: This study has showed that yarrow herbal dust represent a valuable by-product of food industry, in mean high concentration of bioactive compounds, especially aromatic ones e.g. eucalyptol and camphor. Application of supercritical carbon dioxide extraction could be the solution for such material utilization. In extraction process parameters impacted on extraction efficiency, therefor for higher process efficiency and better product/extract quality higher pressures e.g. 300 bar should be applied.

GREEN EXTRACTION OF AGRICULTURAL WASTE: MICROWAVE ASSISTED EXTRACTION OF GRAPEVINE GREEN LEAVES AND STEMS

Aleksandra Gavarić¹, Robert Radosavljević¹, Zorica Drinić¹, Branimir Pavlić¹, Mire Zloh², Bojan Konstantinović³, Senka Vidović¹

¹ University of Novi Sad Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia

² University of Hertfordshire, United Kingdom

³ University of Novi Sad Faculty of Agriculture, Department for Environmental and Plant Protection, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

*Corresponding author: senka.curcin@yahoo.com

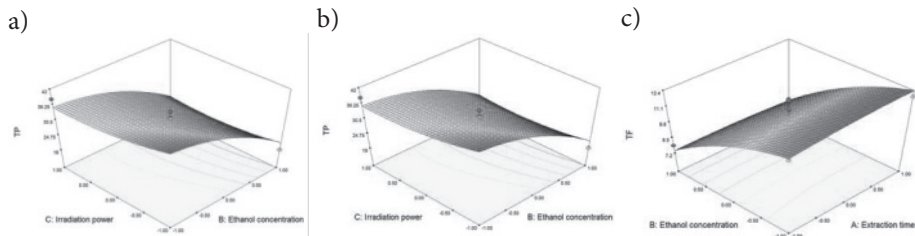
KEYWORDS: grapevine leaves; grapevine stems; agricultural waste; microwave assisted extraction; optimization

INTRODUCTION: During the production of grapes, vineprunings (green leaves and stems) are produced as agricultural waste. Green leaves and stems are discarded partly before blooming and during growth in order to improve the grape harvest and sweetness of the fruit. Considering poorly developed management of vineprunings, this raw material is usually left in or near of vineyards to degrade. Therefore, there is an aspiration to use this waste as resource of more value added products.

OBJECTIVES: The objectives of the present study were to investigate the effects of microwave assisted extraction (MAE) parameters (extraction time, ethanol concentration and irradiation power) on process efficiency and quality of obtained extracts.

METHOD / DESIGN: In this study MAE was applied as novel extraction technique which provides significantly shorter extraction time in comparison to classical processes of extraction. Phytochemical profile of obtained extracts was determined by spectrophotometric measuring of total phenolic (TP), total flavonoid (TF) contents and IC₅₀ and EC₅₀ values. Three level, three variables Box-Behnken design was applied. Extraction time (t, 15-35 min), ethanol concentration (c, 40-80 %) and irradiation power (P, 400-800 W) were independent variables. Experimental values were fitted to a second order polynomial model and multiple regression coefficients were provided for five responses using the method of least square.

RESULTS:



T5

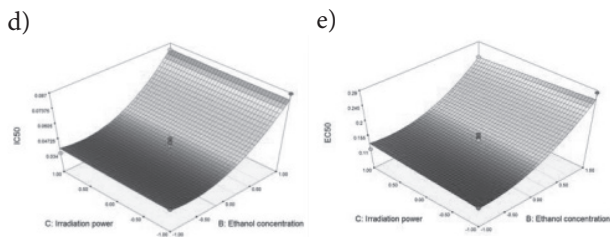


Fig.1. Response surface 3D plots showing combined effects of extraction time, ethanol concentration and irradiation power on: a) Y, b) TP, c) TF, d) IC50 and e) EC50 value.

CONCLUSIONS: Mathematical models, for all five responses, were statistically accepted due to significant regression for the model ($p < 0.05$). The most efficient extraction conditions were extraction time of 35 min, ethanol concentration of 41% and irradiation power of 400 W, while predicted values of Y, TP, TF, IC50 and EC50 at this experimental point were 14.65 %, 40.19 mg GAE/g, 12.77 mg CE/g, 0.0327 mg/mL and 0.1196 mg/mL, respectively.

LARGE-SCALE SOIL MOISTURE MONITORING USING MICRO UNMANNED AERIAL VEHICLE: GUMBASA IRRIGATION SYSTEM CASE STUDY

Nugroho T.W.¹, Jabal T.I.², Wahono³, Sitti, R.M⁴

¹ Department of Forestry, University of Muhammadiyah Malang, Indonesia,

² Department of Agribusiness, University of Muhammadiyah Malang, Indonesia,

³ Department of Agrotechnology, University of Muhammadiyah Malang, Indonesia,

⁴ Department of Agribusiness, University of MuhammadiyahKendari, Indonesia.

*Corresponding author: triwaskithon@yahoo.co.id

KEYWORDS: monitoring; soil moisture; unmanned aerial vehicle.

INTRODUCTION: Soil moisture is an important variable which limiting food security. There are many methods has been studied in monitoring soil moisture at large scale. The development of such monitoring methods has been reviewed intensively. There are three main methods, i.e. (i) in situ and proximal sensing techniques, (ii) dedicated soil moisture remote sensing missions, (iii) soil moisture monitoring networks. All of these techniques need advanced tools and management to be applied. The new development of UAV rises a new hope to monitor farm land soil moisture spatially and economically.

OBJECTIVES: This study is aimed to evaluate the possibility of UAV based application in monitoring farm land moisture.

METHOD / DESIGN: The method consist of data collecting, calibration and data analysis. The UAV platform used in this study is developed based on Farm Mapper Lite. We used a modified action camera (Xiaomi Yi with 16 Mpix CMOS sensor) with 24 mm focal length. Measurement of soil moisture is used DM-15.

RESULTS:



Result of calibration of soil moisture is $Y = 6,946 + 2,512X$

CONCLUSIONS: The conclusion is micro UAV has potential in monitoring soil moisture for large land.



PL- Plenary lectures
 IL - Invited lecturers
 O - Oral presentation

PLENARY LECTURES

PL-1	<p>Giacinto Bagetta¹, Tsukasa Sakurada², Maria Tiziana Corasaniti³, Shinobu Sakurada⁴: RATIONAL BASIS FOR TRANSLATION OF BERGAMOT ESSENTIAL OIL AS NEUROTHERAPEUTIC</p> <p>¹Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, Rende, Italy; ²Drug Innovation Center, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan; ³Department of Health Sciences, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; ⁴Department of Physiology and Anatomy, Tohoku Pharmaceutical University, Sendai, Italy</p>
PL-2	<p>Souwalak Phongpaichit¹, Vatcharin Rukachaisirikul², Jariya Sakayaroj³: BIOACTIVE FUNGAL METABOLITES</p> <p>¹Natural Product Research Center of Excellence and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand, ²Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand, ³National Center for Genetic Engineering (BIOTEC), Thailand Science Park, Klong Luang, Pathumthani 12120, Thailand</p>
PL-3	<p>Luciano Saso: MODULATION OF OXIDATIVE STRESS AS A PHARMACOLOGICAL STRATEGY</p> <p>Faculty of Pharmacy and Medicine, Sapienza University of Rome, Rome, Italy</p>
PL-4	<p>Marco Dalla Rosa: INNOVATION IN FOOD SCIENCE AND TECHNOLOGY AS A SOCIAL AND ENVIRONMENTAL CHALLENGE</p> <p>Alma Mater Studiorum, Università di Bologna, Italy</p>

T1 Biodiversity and Environment

T1 - IL- 1	<p>Sara Bumrungsri¹, Kitichate Sridith¹, Paul Racey²: ECOSYSTEM SERVICES OF BATS IN THAILAND, A CASE STUDY</p> <p>¹Department of Biology, Prince of Songkla University, ²University of Exeter, UK</p>
T1 - IL- 2	<p>Snežana Radenković¹: DIVERSITY AND CONSERVATION OF HOVERFLIES (INSECTA: DIPTERA: SYRPHIDAE) IN SOUTHEAST EUROPE</p> <p>¹Department of Biology and Ecology, Faculty of Sciences, 2 Trg Dositeja Obradovića, 21000 Novi Sad, Serbia</p>
T1 - IL- 3	<p>Anchana Prathep¹, Piyalap Tuntiprapas¹: SEAGRASS BED AS A CARBON SINK IN RANONG BIOSPHERE RESERVE AND TRANG-HAAD CHA MAI MARIN NATIONAL PARK: AN IMPORTANT ROLE OF SEAGRASS</p> <p>Seaweed and Seagrass Research Unit, Excellence Centre for Biodiversity of Peninsular Thailand, Department of Biology, Faculty of Science, Prince of Songkla University, HatYai, Songkhla, Thailand 90112</p>

T1 - IL - 4	<p>Milivoje Krvac¹: EXPLOITATION OF THREATENED NON - CITES AND NEW DISCOVERED REPTILE SPECIES</p> <p>¹<i>Institute for Nature Conservation of Serbia, Dr Ivana Ribara 91, 11070 Belgrade, Serbia</i></p>
T1 - O - 1	<p>Boris Obrovski¹, Ivana Mihajlović¹, Jovan Bajić², Branislav Batinić², Miloš Živanov², Mirjana Vojinović Miloradov¹: DETERMINATION OF KEY PHYSICO-CHEMICAL PARAMETERS OF WATER BODIES QUALITY BY FIBER OPTIC SENSORS (FOS)</p> <p>¹<i>University Of Novi Sad, Faculty Of Technical Sciences, Department Of Environmental Engineering, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia</i></p> <p>²<i>University Of Novi Sad, Faculty Of Technical Sciences, Department of Power, Electronic and Telecommunication Engineering, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia</i></p>
T1 - O - 2	<p>Maja Novković¹, Đorđe Obradović², Dušanka Cvijanović¹, Ivana Krtolica², Aleksandar Kaplar², Milica Živković¹, Ivana Teodorović¹, Mirjana Vojinović-Miloradov², Snežana Radulović¹: BEING SMART IN A MULTI-STRESSED WORLD - PREDICTING THE STRESS IMPACT ON <i>Lemna minor</i> L., <i>Ceratophyllum demersum</i> L. AND <i>Mentha aquatica</i> L. ALONG THE DANUBE RIVER USING ANN TRAINED ALGORITHMS</p> <p>¹ <i>University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology,</i></p> <p>² <i>University of Novi Sad, Faculty of Technical Sciences* Scholar of the Ministry of Education, Science and Technological Development of the Republic of Serbia</i></p>
T1 - O - 3	<p>Dragana Čučak¹, Slavica Perić², Ivica Tamaš², Dragan Radnović²: MICROBIOLOGICAL WATER QUALITY OF SELECTED SURFACE WATERS IN VOJVODINA</p> <p>¹ <i>University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia,</i></p> <p>² <i>University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia</i></p>
T1 - O - 4	<p>Maja Lončarski¹, Jovana Blitvin¹, Aleksandra Tubić¹, Jelena Molnar Jazić¹, Snežana Maletić¹ Srđan Rončević¹, Jasmina Agbaba¹: REMOVAL OF SELECTED PESTICIDES BY MAGNETIC AND/OR MACROPOROUS ION EXCHANGE RESINS</p> <p>¹<i>University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, Trg Dositeja Obradovića 3, 21000 Novi Sad, R. Serbia</i></p>
T1 - O - 5	<p>Milica Stanković¹, Anchana Prathep¹: SEAGRASS BIOMASS MAPPING TECHNIQUES IN ANDAMAN COAST OF THAILAND</p> <p>¹<i>Seaweed and Seagrass Research Unit, Department of Biology, Faculty of Science, Prince of Songkla Univeristy, Hat Yai, Thailand</i></p>
T1 - O - 6	<p>Miljan Šunjević^{1,2}, Mirjana Vojinović-Miloradov², Darko Reba¹: INFULENCE OF URBANISATION OF FRUŠKA GORA ON ENVIRONMENT AND BIODIVERSITY</p> <p><i>University of Novi Sad, Faculty of Technical Sciences, ¹Depatament of Architecture and Urbanism, ²Departament of Environmental Engineering and Occupational Safety and Health</i></p>

T1 - O - 7	Marko Djuracic¹ : TEA WITH GODOT – THE EMERGENCE OF THE PHYLOMORPHOGEORAPGY AS A CONCEPTUALLY VALID METHOD FOR THE SUBSPECIES INFERENCE <i>¹University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology</i>
T1 - O - 8	Dragana Ostojić ¹ , Vladimir Nikolić¹ , Aleksandar Dragišić ¹ : FOREST RESERVES IN SERBIA - THE STATUS, DISTRIBUTION AND SYNECOLOGY <i>¹Institute for Nature Conservation of Serbia, Belgrade, Serbia</i>

T2 Physiology of Living Organisms

T2 - IL - 1	Nebojša Andrić¹ : ENDOCRINE DISRUPTORS AND REPRODUCTION: MECHANISMS OF ACTION IN STEROID HORMONE-PRODUCING CELLS <i>¹University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia</i>
T2 - O - 1	Bojana Stanić¹ , Jelena Hrubik ² , Vivian Lu Tan ³ , Kristin Schirmer ³ , Nebojsa Andrić ² : ESTABLISHMENT OF CELL-BASED ELISA FOR QUANTITATIVE MEASUREMENT OF ENVIRONMENTAL CHEMICAL-INDUCED ERK1/2 ACTIVATION IN HEPG2 CELLS <i>¹UNS FTN, Department of Environmental Engineering and Occupational Safety and Health, Novi Sad, Serbia, ²UNS PMF, Department of Biology and Ecology, Novi Sad, Serbia, ³Eawag, Department of Environmental Toxicology, Dübendorf, Switzerland</i>
T2 - O - 2	Tanja Bulat¹ , Roman Smidak ¹ , Fernando J. Sialana ¹ , Gangsoo Jung ¹ , Thomas Ratte ² , Martin Bilban ³ , Helmut Sattmann ⁴ , Gert Lubec ⁵ , Jana Aradska ¹ : TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF <i>Arion vulgaris</i> — PROTEINS FOR PROBABLY SUCCESSFUL SURVIVAL STRATEGIES? <i>¹Department of Pediatrics, Medical University of Vienna, Vienna, Austria, ²Division of Computational System Biology, Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria, ³Department of Laboratory Medicine and Core Facility Genomics, Medical University of Vienna, Vienna, Austria, ⁴Third Zoological Department, Museum of Natural History Vienna, Vienna, Austria, ⁵Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria</i>
T2 - O - 3	Milan Borišev¹ , Milan Župunski ¹ , Slobodanka Pajević ¹ , Rita Horak ³ , Andrej Pilipović ² , Nataša Nikolić ¹ , Danijela Arsenov ¹ , Saša Orlović ² : DAILY DYNAMICS OF BEECH PHOTOSYNTHETIC PARAMETERS IS SIGNIFICANTLY DISTURBED BY PERIODICAL DROUGHT AND SPECIFIC MICROHABITAT CONDITIONS <i>¹University of Novi Sad, Department of biology and ecology, Faculty of Sciences, Serbia; ²University of Novi Sad, Institute of Lowland Forestry and Environment, Serbia ³University of Novi Sad, Faculty of Education on Hungarian language, Subotica, Serbia</i>

T3 Biotechnology, Bioengineering and Biosensing

T3 - IL- 1	<p>Wilaiwan Chotigeat^{1,2}, Monwadee Wonglapsuwan^{1,2}, Kunwadee Palasin^{1,2}: EXTRA-FUNCTION OF RIBOSOMAL PROTEIN L10A <i>¹Department of Molecular Biotechnology and Bioinformatics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand, ²Center for Genomics and Bioinformatics Research Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla</i></p>
T3 - IL- 2	<p>Vladimir Crnojević¹: BIOSENSE INSTITUTE AND THE FUTURE OF AGRICULTURE - DATA IN ACTION <i>¹BioSense Institute, University of Novi Sad</i></p>
T3 - O - 1	<p>Phutita Wongwaiyut¹, Patamarek Engsontia¹: THE RNAi MACHINERY OF COLLEMBOLA <i>¹Molecular Ecology and Evolution Research Unit, Department of Biology, Prince of Songkla University, Hat Yai, Songkhla 90110 Thailand</i></p>
T3 - O - 2	<p>Darjana Ivetić, Tatjana Đorđević, Mirjana Antov: DETERMINATION OF SOME ELEMENTS FOR TECHNO-ECONOMIC ENZYMATIC HYDROLYSIS OF CELLULOSE IN HYDROTHERMALLY PRETREATED SUGAR BEET SHREDS <i>Faculty of Technology, University of Novi Sad, Blvd. Cara Lazara 1, 21 000 Novi Sad, Serbia</i></p>
T3 - O - 3	<p>Zorana Rončević, Ida Zahović, Bojana Bajić, Jovana Grahovac, Jelena Dodić: XANTHAN BIOSYNTHESIS IN LABORATORY BIOREACTOR ON EFFLUENTS FROM WHITE WINE PRODUCTION <i>Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia</i></p>
T3 - O - 4	<p>Ivana Kovacic¹, Dragi Radomirovic², Miodrag Zukovic¹, Pavel Benka², Milutin Nikolic¹: BIOMECHANICAL AND BIOMIMETICAL PARAMETRIZATION OF TRUNK-DOMINATED TREES <i>¹University of Novi Sad, Faculty of Technical Sciences; ² University of Novi Sad, Faculty of Agriculture</i></p>

T4 Bioactive Natural products-Biochemistry and Pharmacology

T4 – IL - 1	<p>Wilawan Mahabusarakam^{1,3}, Suwanna Deachathai¹, Parichat Thepthong¹, Arun Saelee¹, Pattama Mecawun¹, Ilham Abdullah¹, Souwalak Phongpaichit^{2,3}: <i>Garcinia dulcis</i> Kurz: SECONDARY METABOLITES AND BIOLOGICAL ACTIVITY</p> <p>¹Department of Chemistry, ²Department of Microbiology, ³Natural Product Research Center of Excellence, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand</p>
T4 – IL - 2	<p>Olga Tzakou: ESSENTIAL OILS AS GREEN PESTICIDES</p> <p>Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupoli Zographou, 157 71 Athens, Greece</p>
T4 – IL - 3	<p>Jurathip Wungsintaweekul^{1,2}, Charoenwong Premprasert¹, Supinya Tewtrakul^{1,2}: CYCLOOXYGENASES AND INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITORY ACTIVITIES OF DITERPENES FROM <i>Croton stellatopilosus</i></p> <p>¹Department of Pharmacognosy and Pharmaceutical Botany, ²Phytomedicine and Pharmaceutical Biotechnology Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand</p>
T4 – IL - 4	<p>Nongyao Sawangjaroen¹, Kruawan Hounkong¹, Wipapan Kongyen², Vatcharin Rukachaisirikul³: <i>Coptosapelta flavescens</i> Korth. AS A POTENTIAL ANTI-Giardial COMPOUNDS SOURCE</p> <p>¹Department of Microbiology, Faculty of Science, and Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand, ²Chemistry and Applied Chemistry Program, Faculty of Science and Technology, Songkhla Rajabhat University, Mueang, Songkhla 90000, Thailand, ³Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand</p>
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T4-O-P-2	<p>Isidora Samojlik¹, Biljana Božin², Neda Gavarić², Vesna Mijatović¹, Stojan Petković³: IN VITRO AND IN VIVO ANTIOXIDANT POTENTIAL OF ESSENTIAL OIL OF ANISEED (<i>PIMINELLA ANISUM L. APIACEAE</i>)</p> <p>¹University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology, ²University of Novi Sad, Faculty of Medicine, Department of Pharmacy, ³University of Novi Sad, Faculty of Medicine, Department of Forensic Medicine, Novi Sad, Serbia</p>
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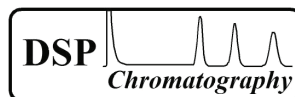
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ANALYSIS d.o.o.
Gandijeva 76a, 11070 Novi Beograd; Tel/fax: +381-(0)-11-318-64-46; +381-(0)-11-318-64-48
e-mail: info@analysis.rs; www.analysis.rs

