



REPUBLIC OF BULGARIA
MINISTRY OF EDUCATION
AND SCIENCE

SCIENTIFIC CONFERENCE
**ECOLOGICAL PRODUCTS
FOR HEALTH**

04 – 08 October 2020,
Spa Hotel Kleptuza, Velingrad

ORGANIZERS:



EUROPEAN UNION
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DEVELOPMENT FUND



OPERATIONAL PROGRAMME
SCIENCE AND EDUCATION
FOR SMART GROWTH

SPONSORS:



SCIENTIFIC CONFERENCE

ECOLOGICAL PRODUCTS

FOR HEALTH

**04 – 08 October 2020,
Spa Hotel Kleptuza, Velingrad**

Dear colleagues,

We are very happy to welcome you in Velingrad!

We hope to contribute to the success of the National program

“BioActiveMed” and the Centre of Competence “Clean & Circle“.

Enjoy the warm and friendly atmosphere of our conference!

Welcome to Spa Hotel Kleptuza in Velingrad

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SYNERGY - SCIENCE, TECHNOLOGY, BUSINESS FORCE PROSPERITY

National science program D01-217/30.11.2018, D01-323/18.12.2019
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Project BG05M2OP001-1.002-0019 *"Clean technologies for sustainable environment – water, waste, energy for circular economy", financed by the OP "Science and Education for Smart Growth" 2014-2020*

PROGRAMME

Sunday, 4th October 2020

15:00-18:30 *Registration*

Monday, 5th October 2020

9:00-14:00	<i>Registration</i>
14:00-14:10	Official Opening of the Conference – Prof. DSc. Pavlina DOLASHKA and Prof. Dr. Nartzislav PETROV
14:10- 14:30	Greetings to the Conference: <ul style="list-style-type: none">• Ministry of Education and Science – Minister KRASIMIR VALCHEV,• Ministry of Health – Minister Prof. DSc KOSTADIN ANGELOV• MES - Prof. Dr. IVAN DIMOV and Prof. Dr. KOSTADIN KOSTADINOV• Operational Programme Science and Education for Smart Growth – MARIA DENIZOVA AND GEORGI IVANOV
14:30-14:50	L1. Violeta Blagova-Damova , Velingrad City Hall, <i>WATERS OF VELINGRAD</i>
14:50-15:10	L2. Prof. DSc. Kostadin Kostadinov , Ministry of Education and Science <i>POLICIES AND MEASURES FOR SCIENCE FUNDING IN BULGARIA</i>
15:10-15:30	L3. Prof. DSc. Pavlina Dolashka , National Research Program „BioActiveMed“ <i>INNOVATIVE LOW-TOXIC BIOACTIVE SYSTEMS FOR PRECISION MEDICINE</i>
15:30-16:00	Coffee break
16:30-16:50	L4. Prof. DSc. Yana TOPALOVA , Center of Competence “Clean&Circle” <i>INOVATIVE AND APPLIED POTENTIAL OF CENTRE OF COMPETENCE ”CLEAN TECHNOLOGIES FOR SUSTAINABLE ENVIRONMENT – WATER, WASTE, ENERGY FOR CICLE ECONOMY”</i>
16:50-17:10	L5. Eng. Ilian Iliev , “Infolab” Ltd. <i>LC-ESI-QTOF, HIGH RESOLUTION AND HIGH MASS ACCURACY SPECTROMETRY FOR ONE SHOT ANALYSIS IN ENVIRONMENTAL AND HEALTH CARE PRODUCTS.</i>
17:10-17:30	L6. Assoc. Prof. Dr. Aleksandar Dolashki , “ALEKS 1977“ Ltd <i>NATURAL PRODUCTS, CONTAINING BIOACTIVE COMPOUNDS FROM MUCUS OF GARDEN SNAIL HELIX ASPERSA</i>

Tuesday, 6th October 2020

Session: Ecological environment - water and air

Chairmen of the session : Prof. DSc. Yana TOPALOVA and Assoc. Prof. Dr. Boiko TSYNTSARSKI

9:00-9:15	L1. Assoc. Prof. Dr. Yovana Todorova , Ivaylo Yotinov, Irina Schneider, Elmira Daskalova, Mihaela Belouhova, Nora Dinova, Yana Topalova, <i>WATER FOR DRINKING USE – POSSIBILITIES AND INNOVATIVE TRENDS IN CONTROL</i>
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	<i>AND TREATMENT</i>
9:15-9:30	L2. Assoc. Prof. Dr. Irina Schneider , Nora Dinova, Ivaylo Yotinov, Mihaela Belouhova, Yovana Todorova, Yana Topalova, <i>ASSESSMENT OF DENITRIFICATION AND NITRIFICATION PROCESSES IN WATER TREATMENT TECHNOLOGIES IN WWTP “SADINATA” AND WWTP “KUBRATOVO”</i>
9:30-9:45	L3. Assist. Prof. Dr. Mihaela Belouhova , Nora Dinova, Ivaylo Yotinov, Stilyana Lincheva, Irina Schneider, Yana Topalova, <i>ANAMMOX AND AZOARCUS-THAUERA CLUSTER - NEW INDICATORS FOR MANAGEMENT OF INFILTRATED WATER PURIFICATION IN THE SOLID WASTE TREATMENT PLANT OF SOFIA</i>
9:45-10:00	L4. Dr. Nora Dinova , Mihaela Belouhova, Nora Dinova, Ivaylo Yotinov, Stilyana Lincheva, Irina Schneider, Yana Topalova, <i>FLUORESCENCE IN SITU HYBRIDIZATION FOR DENITRIFYING MICROORGANISMS – AN INNOVATIVE METHOD IN THE CIRCULAR SOLUTIONS FOR WATER RESOURCES RECOVERY IN MUNICIPAL ENTERPRISE FOR WASTE TREATMENT – SOFIA</i>
10:00-10:15	L5. Eng. Radoslav Tonev , Galina Dimova, <i>PARAMETERS OF HEALTH SIGNIFICANCE FOUND IN DRINKING WATER IN BULGARIA</i>
10:15-10:30	L6. Assoc. Prof. Dr. Boyko Tsyntsarski , Ivanka Stoycheva, Georgi Georgiev, Temenuzhka Budinova, Kalina Kotseva, Angelina Kosateva, Nartzislav Petrov, Andrei Sarbu, Anita Radu, Teodor Sandu, <i>PURIFICATION OF WATER AND AIR BY CARBON ADSORBENTS</i>
10:30-10:45	L7. Assist. Prof. Dr. Ivanka Stoycheva , Georgi Georgiev, Boyko Tsyntsarski, Bilyana Petrova, Nartzislav Petrov, <i>CONVERSION OF RDF FUEL TO CARBON ADSORBENT, LIQUID AND GASEOUS COMBUSTIBLE PRODUCTS</i>
10.45-11:30	Coffee break
11:30-11:45	L8. Assist. Dr. Angelina Kosateva , Ivanka Stoycheva, Bilyana Petrova, Boyko Tsyntsarski, <i>RAMAN SPECTROSCOPY AS A POWERFUL TOOL FOR CHARACTERIZATION OF CARBON MATERIALS</i>
11:45-12:00	L9. Assist. Georgi Georgiev , Ivanka Stoycheva, Boyko Tsyntsarski, Bilyana Petrova, Kalina Miteva, Nartzislav Petrov, <i>SYNTHESIS ON NANOPOROUS CARBON FROM RDF BITUMEN MATERIAL</i>
12:00-12:15	L10. Dr. Gloria Issa , Momtchil Dimitrov, Radostina Ivanova, Tanya Tsoncheva, <i>SYNTHESIS AND PHYSICO-CHEMICAL CHARACTERIZATION OF NANO-STRUCTURED MANGANESE OXIDE PROMOTED WITH CERIA</i>
12.15-12.30	L11. Dr. Radostina Ivanova , Gloria Issa, Momtchil Dimitrov, Tanya Tsoncheva, <i>METAL OXIDES BASED CATALYSTS FOR HYDROGEN PRODUCTION</i>
12.30-12.45	L12. Asist. Dr. Yana Ilieva , Lyudmila Dimitrova, Maya M. Zaharieva, Mila Kaleva, Petko Alov, Ivanka Tsakovska, Tania Pencheva, Hristo Najdenski, Ilza Pajeva, <i>CYTOTOXICITY AND MICROBICIDAL ACTIVITY OF ORGANIC SOLVENTS COMMONLY USED FOR STUDYING PLANT EXTRACTS AND THEIR BIOACTIVE COMPOUNDS: A COMPARATIVE STUDY</i>
12:45-14:00	Lunch
14.00-16.00	Poster session

Wednesday, 07th October 2020

Session: Natural health products

Chairmen of the session: *Prof. Dr. Iva Ugrinova and Prof. Dr. Reni Kalfin*

9:00- 9:20	L1. Prof. Dr. Iva Ugrinova , Maria Petrova, Jordana Todorova, Maria Schröder, Aleksandar Dolashki, Lyudmila Velkova, Tsvetina Stoyanova, Pavlina Dolashka, <i>ANTITUMOR ACTIVITY OF BIOACTIVE COMPOUNDS FROM MARINE SNAIL RAPANA VENOSA AND GARDEN SNAIL HELIX ASPERSA AGAINST A PANEL OF HUMAN BREAST CANCER CELL LINES</i>
9:20-9:35	L2. Maria Schröder - PhD student , Maria Petrova, Jordana Todorova, Iva Ugrinova, <i>THE EFFECT OF A NEW SYNTHETIC FERROCENE CONTAINING CAMPHOR SULFONAMIDE ON BREAST CANCER CELL LINES</i>
9:35-9:50	L3. Lazar Lazarov - student MS , Maria Schröder, Iva Ugrinova, <i>EFFECT OF CANNABIDIOL ON CELL LINES H1299, A549, MRC-5</i>
9:50-10:05	L4. Assoc. Prof. Dr. Anastas Gospodinov , <i>THE STUDY OF THE S-PHASE OF THE CELL CYCLE - MOTIVATION AND METHODS</i>
10:05-10:20	L5. Alexander Dushkov - student MS , Maria Schröder, Maria Petrova, Iva Ugrinova, <i>NATURAL MEDICINE: THE SEARCH FOR POTENTIAL ANTITUMOR PROPERTIES AMONG THE MUSHROOMS OF BULGARIA</i>
10:20-10:35	L6. Vladimir Vazharov , <i>THE HEALING MUSHROOMS OF BULGARIA</i>
10:35-11:05	Coffee break
11.05-11:20	L7. Assoc. Prof. Dr. Peicho Petkov , Elena Lilkova, Tsveta Lazarova, Nevena Ilieva, Leandar Litov, <i>MOLECULAR MODELLING OF ANTIMICROBIAL PEPTIDE MIXTURES FROM THE MUCUS OF GARDEN SNAIL</i>
11:20-11:35	L8. Zlatina Vlahova , Alexander Tzintzarov, Jordana Todorova, Iva Ugrinova, <i>STUDYING THE ACTIVITY OF BIOACTIVE COMPOUNDS ISOLATED FROM GARDEN SNAILS (HELIX ASPERSA, HELIX LUCORUM) AND MURINE SNAILS (RAPANA VENOSA) AGAINST VARIOUS HUMAN CANCER CELL LINES</i>
11:35-11:50	L9. Prof. DSc. Yana Topalova , Elmira Daskalova, Neli Zheleva, Mihaela Belouhova, Pavlinka Dolashka, <i>EFFECTS OF INFLUENCE OF PEPTIDE FRACTIONS FROM MUCUS OF CORNU ASPERSUM, ON ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS AND CLOSTRIDIUM PERFRINGENS</i>
11:50-12:05	L10. Prof. Dr. Krassimira Yoncheva , Niko Benbassat, Maya M. Zaharieva, Lyudmila Dimitrova, Alexander Kroumov, Ivanka Spassova, Daniela Kovacheva, Hristo M. Najdenski, <i>ANTIMICROBIAL ACTIVITY OF OREGANO OIL ENCAPSULATED IN CHITOSAN-ALGINATE NANOPARTICLES</i>
12.05-12.20	L11. MSc. Inna Sulikovska , Ivan Iliev, Zdravka Petrova, Katerina Todorova, Ani Georgieva. <i>EHRlich ASCITES CARCINOMA - A MOUSE MODEL OF BREAST CANCER</i>
12.20-12.35	L12. BSc. Elena Ivanova , Ivan Iliev, Inna Sulikovska, Dardana Manga, Katerina

	Todorova, Ani Georgieva, Valeriya Dilcheva, Ivelin Vladov, Svetlozara Petkova, Reneta Toshkova and Pavlina Dolashka, <i>ASSESSMENT OF THE CYTOTOXIC EFFECTS AND ANTIPROLIFERATIVE ACTIVITY OF HEMOCYANINS FROM HELIX LUCORUM, HELIX ASPERSA AND RAPANA VENOSA</i>
12:35-12:50	L13. Eng. Ventsislav Karamfilov, <i>EXCELLENCE IN ROUTINE AND SCIENCE</i> , LKB -Bulgaria ltd.
12:50-14:00	Lunch
14.00-16.00	Poster session 2
19:00-22:00	Welcome Dinner

Thursday, 08th October 2020

Session: Ecological products and processes

Chairmen of the session: *Prof. DSc. Hristo NAJDENSKI* and *Prof. Dr Nikolay Vassilev*

9:00- 9:15	L1. Assist. Asya Daskalova, Lyudmila Velkova, Ventseslava Petrova, Wolfgang Voelter, Pavlina Dolashka, <i>PROTEOMIC ANALYSIS OF INTRACELLULAR EXTRACTS OF SACCHAROMYCES CEREVISIAE TREATED WITH TOXINS IN BOTH LOG AND STATIONARY PHASE</i>
9:15- 9:30	L2. Dr. Nadezhda Kostova, Tsvetelina Doncheva, Kalina Alipieva, <i>METABOLIC PROFILE OF BIOACTIVE COMPONENTS FROM GENTIANA SPECIES</i>
9:30-9:45	L3. Assoc. Prof. Dr. Lyudmila Velkova, Aleksandar Dolashki, Elmira Daskalova, N. Zheleva, Yana Topalova, Ventseslav Atanasov, Wolfgang Voelter and Pavlina Dolashka, <i>INVESTIGATION OF DIFFERENT FRACTIONS FROM MUCUS OF THE GARDEN SNAIL CORNU ASPERSUM WITH ANTIBACTERIAL ACTIVITIES</i>
9:45-10:00	L4. Dimitar Kaynarov, Aleksandar Dolashki, Olga Boyanova, Lyudmila Velkova, Wolfgang Voelter and Pavlina Dolashka, <i>INVESTIGATION OF ANTITUMOR POTENTIAL OF HEMOCYANINS FROM GASTROPODAN AGAINST T24 BLADDER CARCINOMA</i>
10:00-10:15	L5. Assist. Ventseslav Atanasov, Lyudmila Velkova, Lubka Tancheva, Aleksandar Dolashki, Reni Kalfin, Pavlina Dolashka, <i>CORTEX AND HIPPOCAMP PROTEIN PROFILE ON SCOPOLAMINE RAT MODEL OF ALZHEIMER'S DISEASE WITH APPLICATION OF SNAIL EXTRACT FROM HELIX ASPERSA AS NEUROPTOTECTIVE AGENT</i>
10:15-10:45	Coffee break
10:45-11:00	L6. Dr. Momchil Kermedchiev, Irina Michael, Evgenia Peneva, Radka Lazarova, Stela Jordanova, Pavlina Dolashka, <i>A NEW APPROACH IN THE TREATMENT OF DIFFICULT TO HEAL AND CHRONIC WOUNDS</i>

11:00-11:15	L7. Prof. Dr. Lyubka Tancheva , Maria Lazarova, Pavlina Dolashka, Alexander Dolashki, Lyudmila Velkova, Miroslava Stefanova, Diamara Uzunova, Petya Gavrilova, Albena Alexandrova, Elina Tsvetanova, Yordan Hodzev, Reni Kalfin, <i>FRESH EXTRACT OF SNAIL (HELIX ASPERSA) IMPROVES MEMORY CAPACITY IN EXPERIMENTAL MODEL OF DEMENTIA IN RATS</i>
11:15-11:30	L8. Assist. Prof. Dr. Daniel Todorov , <i>VIRUCIDAL ACTIVITY OF DIFFERENT COMPONENTS FROM HEMOLYMPH</i>
11:30-11:45	L9. Prof. Dr. Nikolay Vassilev , Svetlana Simova, Miroslav Dangalov, Lyudmila Velkova, Venceslav Atanasov, Aleksandar Dolashki and Pavlinka Dolashka, <i>NMR SPECTROSCOPY FOR METABOLOMICS RESEARCH</i>
11:45-13:00	Poster session 3
13:00-13:25	Closing ceremony

The reports will be published as articles in a special issue of the Bulgarian Chemical Communications journal

ABSTRACTS - LECTURES



Lecture 1. WATERS OF VELINGRAD

Violeta Blagova-Damova, *Velingrad City Hall*

Velingrad, unique with an exceptional combination of extremely beautiful nature, favorable climatic conditions and abundance of healing mineral waters, is undoubtedly has won renown as the pearl of Bulgarian balneology.

Leading balneologists, hydrologists and climatologists from the National Research Institute of Balneology, Physiotherapy and Rehabilitation in Sofia, Bulgaria, have been conducting detailed studies on Velingrad mineral springs, their balneological characteristics and medical application, and they have justified their indisputable healing properties on the base of scientific research. There are several dissertations defended by doctors working in the resort.

In the last 20 years, dozens of hotels and guest houses have been built in the resort, and there are 4 Balneotherapy (Medical SPA) and 16 SPA centers working, all certified under the Bulgarian Tourism Act. The unique possibilities and features of the resort have received well-deserved recognition by awarding Velingrad in 2005 for SPA capital of Bulgaria, and in 2008 for SPA capital of the Balkans.

In terms of abundance and variety of mineral waters, the resort is ranked first in Bulgaria. The Velingrad mineral water source is the most extensively studied thermal basin in Bulgaria. It is of sillite type, with classical and unique character - classical, due to the fact that it demonstrates almost the entire natural hydro-geochemical range of sillitic acrotherms, and unique, due to the presence of numerous resources (over 80 springs with a total flow of 160 - 170 liters in second) and their unusual concentration in a small area. The main characteristic of the Velingrad waters is that despite the low total mineralization, the available micro-elements, micro-components and gases are completely dissociated in ionic form, which determines their activity and specific beneficial effects on the human organism and health.



REPUBLIC OF BULGARIA
MINISTRY OF EDUCATION
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Lecture 2. POLICIES AND MEASURES FOR SCIENCE FUNDING IN BULGARIA

Prof. Dr. Kostadin Kostadinov, *Ministry of Education and Science*

The importance of the science for development of knowledge based economy and the role of science is in the base of the strategical documents as Research and Innovation strategy for smart innovations and National Strategy for Development of Scientific Research in the Republic of Bulgaria 2017 – 2030. Different measures and instruments for science funding are analyzed in respect of their impact for successful realization of both strategic documents. They are divided on the 3 levels – national, regional and union levels. Every instrument is considered separately and how it could be used in synergy with other instruments to maximize their impact to the national economy and to the implementation of national science policies. In conclusion some recommendations are proposed to the scientists how to use the considered instruments to increase their science excellence and to attract young scientists for development and sustainability of Bulgarian science.



Lecture 3: INNOVATIVE LOW-TOXIC BIOACTIVE SYSTEMS FOR PRECISION MEDICINE

Prof. DSc. Pavlina Dolashka and Asoc. Prof. Dr. Olya Stoilova,
National Research Program „BioActiveMed“

The National Research Programme “*Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)*” was approved by DCM №658/14.09.2018 and is performed on the basis of a signed agreement between Bulgarian Ministry of Education and Science and Bulgarian Academy of Sciences (BAS). A prerequisite for the implementation of the NRP BioActiveMed is the fact that Bulgaria, with its unique climatic and ecological factors, offers an amazing variety of plant and animal species. Isolated extracts contain a series of bioactive compounds that resembles combined therapy with several synthetic compounds. It is well known that extracts from natural products are less toxic than synthetic and are better tolerated by the human body. Therefore, the proposed in the Programme approach is one of the key to overcoming multiple drug resistance.

The leading organization is the Bulgarian Academy of Sciences and the following scientific organizations (seven scientific units from the BAS) and higher education organizations with the highest capacity in the field of the Programme are Partners: Institute of Organic Chemistry with Center of Phytochemistry (Partner 1), Institute of Molecular Biology (Partner 2), Institute of Microbiology (Partner 3), Institute of Neurobiology (Partner 4), Institute of Experimental Morphology, Pathology and Anthropology with Museum (Partner 5), Institute of Polymers (Partner 6), Institute of Information and Communication Technologies (Partner 7), Medical University – Sofia (Partner 8), Sofia University “St. Kliment Ohridski” (Partner 9), Medical University – Plovdiv (Partner 10), Plovdiv University “Paisii Hilendarski” (Partner 11) and National Sports Academy “Vasil Levski” (Partner 12).

Web page of the Programme is: www.bioactivemed-nrp.com.

Keywords: *extracts of natural sources, bioactive compounds, innovative systems, precision medicine, food supplements and cosmetics*

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018, D01-323/18.12.2019) under the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)” approved DCM# 658 / 14.09.2018.



clean & circle

Lecture 4. INOVATIVE AND APPLIED POTENTIAL OF CENTRE OF COMPETENCE ”CLEAN TECHNOLOGIES FOR SUSTAINABLE ENVIRONMENT – WATER, WASTE, ENERGY FOR CICLE ECONOMY”

Prof. DSc. Yana Topalova, Center of Competence “Clean&Circle”

The center of competence Clean&Circle is focused on the investment in a scientific infrastructure, a professional capacity, an innovative technological and business model that create conditions for the functioning of a sustainable ecosystem in the field of the circular economy. This model of production and consumption limits to a minimum the waste, provides a resource and energy effectiveness. In the center the effect will be achieved in four fields: control and technologies for water treatment; treatment, recycling and disposal of solid waste; creation of effective solutions for obtaining renewable and alternative energy sources, materials and resources; transfer and commercialization of

the technologies. The institutional partners are Sofia university "St. Kliment Ohridski" /leading organization/ and University of Architecture, Civil Engineering and Geodesy, University of Forestry – Sofia, "Prof. Dr. Assen Zlatarov" University – Bourgas, Institute of Physical Chemistry "Academician Rostislav Kaishev" - Bulgarian Academy of Sciences, Institute of Organic Chemistry with Center for Phytochemistry - Bulgarian Academy of Sciences, Institute of Microbiology "Stefan Angelov" - Bulgarian Academy of Sciences, "Cleantech Bulgaria" Foundation.

As instance potential of Clean&Circle the two innovative clean technologies are presented. One of them is technology for elimination of toxic /priority/ pollutants in the water treatment plants – critical problem without solution in the practice. The other is productions of biogas with innovations in the three critical moments of the routine biogas production technologies. These innovations resulted more biogas production with higher quality and circular supply green energy. All presented intelligent solutions are on the two levels – molecular methods for control and management and combination of innovations on the technological level to achieve hybrid technologies for circular treatment of waste water and solid biodegradable waste.

Keywords: *center of competence, clean technologies, circular economy, innovative technologies for waste water treatment, technology for biogas production, intelligent biotechnological solutions resulted molecular control and hybrid technologies.*

ACKNOWLEDGEMENTS

This work was supported by Project BG05M2OP001-1.002-0019: 'Clean Technologies for Sustainable Environment - Waters, Waste, Energy for a Circular Economy', financed by Operational Program 'Science and education for smart growth', co-financed by the European Union through the European structural and investment funds.



Lecture 5. LC-ESI-QTOF, HIGH RESOLUTION AND HIGH MASS ACCURACY SPECTROMETRY FOR ONE SHOT ANALYSIS IN ENVIRONMENTAL AND HEALTH CARE PRODUCTS

Eng. Ilian Iliev, Infolab ltd., Sofia, Bulgaria

Bruker LC-ESI-QTOF-MS systems provide cutting edge performance in one-shot analysis for identification and quantitation from small molecules up to antibodies.

It is the first instrument able to provide high resolution and high mass accuracy performance at fast chromatography speeds for the ultimate in sample analysis.

Unique software packages provide additional capabilities to analyze unknowns and give these instruments the power far beyond other instruments in their class.

Three dimensions of information simultaneously raise your analytical tasks to unrivaled heights of confidence: Measure with superior accurate mass Validate with True Isotopic Pattern (TIP) analysis Also benefit from accurate mass and TIP in analysis of fragments in MS/MS mode.

Our Compact II, ESI-QTOF Instant Expertise™ mass spectrometers are the showcase instrument platform for Synthetic chemistry support, Drug metabolite, degradant and impurity identification and quantitation, Biomarker discovery and validation in proteomics and metabolomics, Intact protein analysis and characterization of biopharmaceuticals environmental and health care products.

Powerful Data Analysis software package with support of ProteinScape powered by GlycoQuestDetect glycopeptides in complex LC-MS/MS data sets Identify glycan and peptide moieties Glycan composition and peptide sequence analyzed in one spectrum.

In this presentation, we will make an attempt to review some interesting solutions, involving the analysis of both targeted and unknown compounds in complex matrices for life science research, drug discovery and development, and screening applications.



"ALEKS 1977" Ltd.

**Lecture 6. NATURAL PRODUCTS, CONTAINING BIOACTIVE
COMPOUNDS FROM MUCUS OF GARDEN SNAIL H.
ASPERSA**

**Assoc. Prof. Dr. Aleksandar Dolashki, "ALEKS 1977" Ltd, Sofia,
Bulgaria**

As a result of many years of scientific research and accumulated results, the company "ALEKS 1977" Ltd. was established. It aims to create innovative cosmetic products and food supplements based on studied biocomponents. Our products under the brand name "GOLDEN SNAIL" are developed after a successful research in collaboration with a number of prestigious foreign research laboratories and are based on natural components, such as high-quality purified extract of garden snail *Helix aspersa* and various plant species.

They help speed up tissue regeneration and strengthen the body's immune system. The products do not contain parabens, silicones and artificial colours, so as not to cause allergic reactions. They are suitable for all skin types and for all ages, both for men and women. The unique properties of the developed products are due to the extract collected from the garden snail of the species *Helix aspersa*, which contains extremely important and valuable components such as collagen, elastin, allantoin, glucosamine glycans, proteoglycans, peptides and glycopeptides, glycolic acid, vitamins A, C and E. The active ingredients are very similar in composition and structure to those in the human body, which supports the processes of recovery and regeneration of the skin. Snail extract successfully protects and shields the skin from the harmful effects of the environment, causing premature ageing. The extract is gathered from snails without causing them any suffering or injury, after which they continue their normal lifestyle!

The effectiveness and safety of the products of "ALEKS 1977" Ltd. have been proven through numerous tests that do not use animal experiments.

Products are manufactured in a licensed facility in Sofia, Bulgaria. The facility for production of cosmetic products and food supplements has implemented a certified quality control system in accordance with the requirements of the International standard (GMP and HACCP).

Keywords: snail, cosmetics, food supplement

ACKNOWLEDGEMENTS

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SESSION 1: Ecological environment - water and air

Lecture 1. WATER FOR DRINKING USE – POSSIBILITIES AND INNOVATIVE TRENDS IN CONTROL AND TREATMENT

Assoc. Prof. Dr. Yovana Todorova¹, Assist. Prof. Dr. Ivaylo Yotinov¹, Assoc. Prof. Dr. Irina Schneider¹, Elmira Daskalova¹, Assist. Prof. Dr. Michaela Belouhova¹, Dr. Nora Dinova¹, Prof. DSc. Yana Topalova¹

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Water is an integral component of modern understanding for healthy life and lot of effort is focused on supply of drinking water with high safety and quality. Improving of quality of drinking water can result in strong benefits to health and additional advantages for people. Essential part of drinking water quality is its treatment and control. Achievement of high treatment effectiveness and decontamination of drinking water is crucial question in global water situation to response adequately to challenges of worldwide population growth, climate change and water pollution. The development of new advanced technologies with potential to provide sufficient effect at low costs, timesaving and non-toxicity is attracted the growing scientific interest. Many recently developed technologies are process-based on reverse osmosis, membrane filtration (with new materials on nanoscale), ion exchange, advanced oxidation technologies. One of the technological designs with potential to obtain very efficient bacterial removal is including of plasma modules in treatment. Plasma-based treatment modules are able to produce very high concentrations of energetic and chemically active species (radicals, H₂O₂, O₃, ultraviolet radiation, charged particles, excited metastable atoms and electric fields) with strong germicidal effect on bacteria, spores and bacterial associations. The use of these new advanced methods for treatment and control of drinking water has a potential to increase the quality of drinking water and to contribute to the solution of global water problems.

Keywords: *drinking water, microbial quality, quality, advanced technologies, plasma-based methods*

ACKNOWLEDGEMENTS

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Lecture 2. ASSESSMENT OF DENITRIFICATION AND NITRIFICATION PROCESSES IN WATER TREATMENT TECHNOLOGIES IN WWTP “SADINATA” AND WWTP “KUBRATOVO”

Assoc. Prof. Dr. Irina Schneider¹, Dr. Nora Dinova¹, Assist. Prof. Dr. Ivaylo Yotinov¹, Assist. Prof. Dr. Mihaela Belouhova¹, Assoc. Prof. Dr. Yovana Todorova¹, Prof. DSc. Yana Topalova¹

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The technology on base of denitrification and nitrification (D/N) is widely applicable in biological treatment of municipal and industrial wastewaters as well as of leachate from municipal solid waste landfills. Nevertheless the availability of huge number of scientific reports and practical consideration on this subject, the management of technologies on base of denitrification and nitrification is difficult and always remains critical technological problem. The aim of this study is to

make a comparative assessment between denitrification and nitrification processes in two types of wastewater treatment plants and on this basis to differentiate the key factors for their management. The samples were taken from the biobasins in denitrification and nitrification zone from Wastewater Treatment Plant “Kubratovo” (for treatment of municipal and some industrial wastewaters of Sofia City) and Wastewater Treatment Plant “Sadinata” (for treatment of landfill leachate from municipal solid waste landfill of Sofia City).

The obtained results showed that denitrification was the critical process in the technology on base of denitrification and nitrification. This process is from critical significance for WWTP “Sadinata”. As a major factor that governed the denitrification was the concentration of biodegradable organic matter. The lower organic concentration /measured as COD/ in WWTP “Sadinata” in comparison to WWTP “Kubratovo” led to: decrease of the count of heterotrophic microorganisms, decrease of the total activity of the activated sludge /measured as total dehydrogenase activity/ and inhibition of the nitrate-reductase activity. The nitrification processes in both WWTPs were accomplished with high intensity. The results confirmed that D/N could be optimized and controlled by means of specific for the wastewater treatment technologies parameters and purposely applied indicators.

Keywords: *activated sludge, denitrification and nitrification, microbiological and enzymological control*

ACKNOWLEDGEMENTS

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Lecture 3. ANAMMOX AND AZOARCUS-THAUERA CLUSTER - NEW INDICATORS FOR MANAGEMENT OF INFILTRATED WATER PURIFICATION IN THE SOLID WASTE TREATMENT PLANT OF SOFIA

Assist. Prof. Dr. Mihaela Belouhova¹, Dr. Nora Dinova¹, Assist. Prof. Dr. Ivaylo Yotinov¹, Dr. Stilyana Lincheva², Assoc. Prof. Dr. Irina Schneider, Prof. DSc. Yana Topalova¹,

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The circular economy is in the public focus for couple years now. It encompasses virtually all stages of the products life cycle. In the circular chain special attention is put on the stage where the already used products (the waste) should be transformed into new products with new value. The latter is mainly performed in waste treatment plants and in wastewater treatment plants. It often includes biotechnological processes which are complex and thereafter with largely unknown nature. Because of this their control is very difficult. The specialists on site need new control mechanisms and indicators to raise the efficiency of the processes. This is especially true when it comes to the treatment of the landfill leachate. In the presented study the potential of two unconventional bacterial groups as microbiological indicators for the leachate treatment process in Municipal enterprise for waste treatment (MEWT) was investigated. Two model aerobic bioreactors with two types of activated sludge were used – the one was from MEWT and the other – from Sofia wastewater treatment plant (WWTP). The COD (chemical oxygen demand) and SVI (sludge volume index) were monitored during the 21 days of functioning. The quantity and the spatial distribution of the ANAMMOX and *Azoarcus-Thauera* were investigated with fluorescence *in-situ* hybridization and digital analysis of the obtained images. The data showed that in the end of the process, when the COD was 2317.85 mgO₂/L and SVI was just 4.27 mL/g, the ANMMOX bacteria were 2,5 times more in the activated sludge from MEWT than in this from WWTP. The structure of their population was with bigger clusters with high fluorescence intensity showing higher metabolic activity. The bacteria belonging to the *Azoarcus-Thauera* cluster were also more in the MEWT activated sludge with 10,5 times. The registered significant abundance of the two microbial groups in MEWT compared to the Sofia WWTP is related with the adaptation of the activated sludge which originally

treats the landfill leachate. The obtained results show that the two bacterial groups, which weren't monitored in the MEWT until now, are promising indicators for the ANAMMOX and denitrification processes.

Keywords: ANAMMOX, *Azoarcus-Thauera*, activated sludge, fluorescence in-situ hybridization, activated sludge, circular economy

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Lecture 4. FLUORESCENCE *IN SITU* HYBRIDIZATION FOR DENITRIFYING MICROORGANISMS – AN INNOVATIVE METHOD IN THE CIRCULAR SOLUTIONS FOR WATER RESOURCES RECOVERY IN MUNICIPAL ENTERPRISE FOR WASTE TREATMENT - SOFIA

Dr. Nora Dinova¹, Assist. Prof. Dr. Mihaela Belouhova¹, Assist. Prof. Dr. Ivaylo Yotinov¹, Dr. Stilyana Lincheva², Assoc. Prof. Dr. Irina Schneider¹, Prof. DSc. Yana Topalova¹

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The achievement of circular economy and sustainable development requires waste minimisation and the continual use of resources. New technologies and innovative control systems have to be developed and implemented to contribute to this aim.

The control system used in the management of the resource recovery is really important. When talking for biotechnologies, the choice of the right methods and indicators is a key moment to be assured exact and fast results reflecting the functioning of the biological systems.

The fluorescence *in situ* hybridization is an innovative method that gives the opportunity not just to quantify the identified microorganisms but also to localize them. This gives the priority to get deeper in the relationships between the microorganisms and to extract more information about their functioning and the biological processes at a whole.

The fluorescence *in situ* hybridization have been used in the control systems in the management of different resource recovery technologies by the team of this study. One of its case studies is the Municipal enterprise for waste treatment – Sofia. This enterprise includes several technologies for recovery of different resources – waste, waters and energy. The fluorescence *in situ* hybridization for different groups of microorganisms (*Pseudomonas sp.*, *Acinetobacter sp.*, domain Archaea, *Methanosaeta sp.*, *Methanosarcina sp.*, family *Methanobacteriaceae*, etc.) have been proved its large potential as a method used in the management of such kind of technologies. The last indicators developed by the team are the quantity and localization of denitrifying microorganisms (*Alcaligenes sp.*, *Paracoccus sp.*) defined by fluorescence *in situ* hybridization in a model biotechnology for landfill leachate treatment.

Keywords: circular economy, FISH, innovative method, resource recovery, waste situ hybridization in the management of the resource recovery in waste treatment plants.

ACKNOWLEDGEMENTS

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Lecture 5. PARAMETERS OF HEALTH SIGNIFICANCE FOUND IN DRINKING WATER IN BULGARIA

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According to the National Report of the Executive Environment Agency (EEA)ⁱ, *Microbiological parameters, Nitrates, Chromium and Uranium* are parameters of health significance for which however there are frequent deviations in drinking water in some regions in Bulgaria. At the same time, there is no drinking water treatment plant in Bulgaria for removing those chemical parameters. The paper will make analyses of the health risk associated with these parameters, the regions with persistent deviations of these health significant parameters, as well will propose measures at national and regional level to overcome these problems.

The presentation makes an overview of the health significant parameters for which are observed frequent deviations in the drinking water in some water supply systems in Bulgaria. The level and frequency of exceedance are analyzed, based on monitoring data within the requirements of Ordinance No 9ⁱⁱ on the quality of water intended for drinking and household purposes, executed by the Regional Health Inspectorates and the Regional Water Supply Operators. The associated health risks are presented together with recommendations on how to overcome these deficiencies in the drinking water quality in a sustainable way.

Keywords: *drinking water quality, health significant parameters, quality standards*

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Lecture 6. PURIFICATION OF WATER AND AIR BY CARBON ADSORBENTS

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Activated carbons are effective adsorbents for removal of undesirable organic and inorganic pollutants from drinking water, in the treatment of industrial waste water; air purification of rooms and respirators. Activated carbons are used in medicine and health applications for bacteria and virus removal, adsorptive removal of certain toxins and poisons, purifications of blood, etc.

More than 800 specific organic and inorganic chemical compounds have been identified in drinking water. These compounds are derived from industrial and municipal discharge, natural decomposition of vegetable and animal matter, and from water and waste water chlorination practices. Many of these liquid effluents are carcinogenic. Several methods such as coagulation, oxidation, aeration, ion exchange, and activated carbon adsorption have been used for the removal of these chemical compounds. Many studies including laboratory tests and field operations have indicated that the activated carbon adsorption is perhaps the best method for water purification.

The adsorption capacity of an activated carbon for metal cations from the aqueous solutions depends on surface area, pore size distribution, carbon surface, and the nature of the metal ions in the solution. Other important parameters which influence the adsorption from aqueous solutions are the carbon-oxygen functional groups on the carbon surface and the pH of the solution.

The adsorption of metal ions mainly involves electrostatic attractive and repulsive interactions between metal ionic species in the solution and the negative sites on the carbon surface produced by the ionization of acidic groups.

In the adsorption of organics, however, the situation is quite different. The organic compounds present in water can be polar or nonpolar, so that not only electrostatic interactions but also dispersive interactions will play an important role. In addition, the molecular dimensions and hydrogen bonding is also an important consideration in the adsorption of organic molecules.

Keywords: *Water purification; activated carbon; porosity; surface groups; adsorption*

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Lecture 7. CONVERSION OF RDF FUEL TO CARBON ADSORBENT, LIQUID AND GASEOUS COMBUSTIBLE PRODUCTS

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Composition RDF is a mixture of materials, characterized by a higher combustibility (e.g. paper, plastic) as compared with the components in the total waste stream. RDF is successfully used as a fuel additive in coal-fired boilers (calorific value between 12,000 and 16,000 J / g) in thermal power plants or as a stand-alone fuel for specially designed facilities. A big problem with thermal power plants is the high content of hazardous organic substances in pyrolysis gases. This requires their purification as this are commonly used filters containing a carbon adsorbent.

The aim of the research is to develop a method for processing RDF fuel, which leads to production of liquid and gaseous combustible products and solid product with carbon adsorbent properties are obtained. RDF fuel from waste tarpaulin made of polyvinyl chloride was tested. As a result of the treatment, a nanoporous material with a high surface area of 600-700 m² was obtained.

The initial material was examined by TG and DSC analysis. The resulting carbon adsorbent was characterized by Elemental Analysis, J₂ Adsorption, Nitrogen Porosimetry. The obtained results show that the material has a potential application for adsorption of contaminants.

Keywords: *RDF fuel, waste materials, adsorbent*

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Lecture 8. RAMAN SPECTROSCOPY AS A POWERFUL TOOL FOR CHARACTERIZATION OF CARBON MATERIALS

Dr. Angelina Kosateva¹, Dr. Ivanka Stoycheva¹, Dr. Bilyana Petrova¹, Assoc. Prof. Dr. Boyko Tsyntsarski¹

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Raman spectroscopy was used to study several carbon samples. The study demonstrates the unique ability of Raman spectroscopy to detect structural changes during the activation of the carbon materials. This allows to be studied the changes in the structure and the formation of the porous texture as a result of the physical activation of the carbon materials which significantly determine

their applicability in the purification industry.

Carbon materials have different industrial applications. Activated carbons are unique and versatile adsorbents - they are applied for removal of undesirable odor, color, taste, and other organic and inorganic impurities from domestic and industrial waste water, solvent recovery; in the removal of color from various syrups and pharmaceutical products; in air pollution control from industrial and automobile exhausts; in the purification of many chemical, pharmaceutical, and food products.

Activated carbon materials are effective in removal of organic and inorganic pollutants from water, due to their high surface area and micro-meso porosity, as well as simple design and easy operation of activated carbon adsorption process.

Keywords: *Raman spectroscopy, carbon materials, activated carbons*

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Lecture 9. SYNTHESIS ON NANOPOROUS CARBON FROM RDF BITUMEN MATERIAL

Eng. Georgi Georgiev¹, Dr. Ivanka Stoycheva¹, Assoc.Prof.Dr. Boyko Tsyntsarski¹, Dr. Bilyana Petrova¹, Eng. Kalina Miteva¹, Prof. Dr. Nartzislav Petrov¹

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Refuse-derived fuel (RDF) is a fuel produced from various types of waste such as municipal solid waste and industrial waste. RDF consists of combustible components, like polymers, paper and other waste materials. RDF can be also produced from used tyres or biomass waste. The combusted biomass fraction of RDF is used by stationary combustion operators to reduce their overall reported CO₂ emissions.

Nanoporous carbons is prepared from RDF from waste tarpaulin made of bitumen by pyrolysis at 600°C and subsequent hydro-pyrolysis at 800°C. The synthesized carbon was characterized by N₂ physisorption at -196 °C, determination of oxygen-containing surface groups, IR spectroscopy, etc.

The results show that nanoporous carbon from RDF is characterized by high surface area of 700 m²/g and significant content of micro- and mesopores. The results suggest that obtained nanoporous carbon material is suitable for application as effective adsorbent of organic and inorganic pollutants.

Keywords: *RDF fuel, waste materials, adsorbent*

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Lecture 10. SYNTHESIS AND PHYSICOCHEMICAL CHARACTERIZATION OF NANOSTRUCTURED MANGANESE OXIDE PROMOTED WITH CERIA

Dr. Gloria Issa¹, Assoc. Prof. Dr. Momtchil Dimitrov¹, Dr. Radostina Ivanova¹, Prof. DCs Tanya Tsoncheva¹

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Manganese oxide-based (MnO_x) catalysts have received increased attention due to their low cost, low toxicity, and the ability to degrade organic molecules at low temperatures.

The aim of current investigation is to study the effect of cerium addition of mesoporous nanostructured MnO_x-based catalysts on their catalytic behaviour in ethyl acetate oxidation as a member of VOCs. For the purpose, a series of nanostructured MnO_x-based catalysts were prepared by a co-precipitation of the initial metal nitrates (CP) and by co-precipitation of the metal chlorides with ammonia in the presence of surfactant (CTAB) (HT). Bi-component CeMn materials were obtained through the same techniques as the ratio Ce/Mn was 1:1. A wide arsenal of analytical techniques, such as nitrogen physisorption, XRD, H₂-TPR, Raman, TEM, and XPS spectroscopies, were applied for their characterization. The total oxidation of ethyl acetate was studied in a flow type reactor using gas chromatograph for analyses. Spectral analyses indicated presence of MnO_x species which type and amount vary depending on the preparation method used. It can be concluded that the varying abundance of redox-active Mn²⁺/Mn³⁺/Mn⁴⁺ surface sites and the high proportion of oxygen species together with the particle dispersion, and morphology are significant to its high catalytic activity for the catalytic oxidation of ethyl acetate. For mixed oxide, partial substitution of manganese within the ceria fluorite lattice was detected. Higher catalytic activity for the binary CeMn oxides in comparison with pure manganese oxide materials was observed. The physicochemical study indicated that it is promoted by the stabilization of more finely dispersed MnO_x species in close interaction with CeO₂. The XPS, TPR, and Raman analyses demonstrated that the contact between different metal oxide particles creates an interface layer, where manganese ions in different oxidation state are penetrated into the ceria lattice with simultaneous formation of oxygen defects.

Keywords: *Nanostructured manganese oxide-based catalyst, binary Ce-Mn oxides physicochemical characterization, catalytic oxidation of ethyl acetate*

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Lecture 11. METAL OXIDES BASED CATALYSTS FOR HYDROGEN PRODUCTION

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In the age of mobile technology, energy storage is becoming an extremely important issue, as energy sources such as fossil fuels and natural gas are not available, and even if they were, their combustion or the formation of by-products is not environmentally friendly. Hydrogen fuel is a promising alternative to petroleum-based liquid fuels, as it offers benefits including near-zero pollutant emissions, a diverse primary energy source, high energy conversion efficiency, and the only by-product is water. Hydrogen is the cleanest fuel, but a serious challenge is to find new, cost-effective and highly efficient approaches to energy production, supply and use that will improve the quality of life and will not endanger the environment. In this regard, the usage of methanol is one of the common approaches for the production of hydrogen, which is carried out with the use of a reformer, which produces CO, CO₂ and H₂. The process of methanol decomposition offers serious prospects due to the high capacity of hydrogen production, due to the need to introduce a small amount of heat and the absence of oxidizing agents or steam flow. The improvement of the catalytic processes, as well as the development of new types of catalysts are the major approach for improving the efficiency of the chemical, petrochemical and refining industries, which can be used to achieve sustainable environmental protection. The need of production of cheap and efficient catalysts requires the usage not only of low cost supports but also the replacement of the expensive noble metals by transition metals based active substances. The main disadvantage of the latter is their significantly lower catalytic activity but nanosized bi-metallic materials provide promising solution of this problem. It has been demonstrated that nanostructured composite materials consisting of two or more components in different ratios reveal practically unlimited possibilities for optimizing the properties

of catalysts by development of mixed oxide structures, which can regulate their dispersion, redox and catalytic properties.

Keywords: *methanol decomposition, hydrogen production, transition metal oxides, alternative ecological fuel*

ACKNOWLEDGEMENTS

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Lecture 12. CYTOTOXICITY AND MICROBICIDAL ACTIVITY OF ORGANIC SOLVENTS COMMONLY USED FOR STUDYING PLANT EXTRACTS AND THEIR BIOACTIVE COMPOUNDS: A COMPARATIVE STUDY

Asist. Dr. Yana Ilieva¹, Assist. Prof. Dr. Lyudmila Dimitrova¹, Assist. Prof. Dr. Maya M. Zaharieva¹, Mila Kaleva¹, Petko Alov², Assoc. Prof. Dr. Ivanka Tsakovska², Prof. Dr. Tania Pencheva², Prof. DSc. Hristo Najdenski¹, Prof. DSc. Ilza Pajeva²

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Aim of the study is to evaluate the cytotoxicity and antimicrobial capacity of seven organic solvents commonly used for studying plant extracts and their bioactive compounds on a panel of non-tumorigenic (CCL-1, HaCaT, hTCEpi) and tumorigenic (A-375, A-431) cell lines, bacterial pathogens and the fungus species *Candida albicans* and to illustrate the importance of solvent selection by comparing the cytotoxicity of cranberry proanthocyanidins dissolved in various solvents. The microbicidal effect and the bacterial respiratory activity were evaluated by MBD and MTT tests, respectively. The median inhibitory concentrations (IC₅₀) were tested with the MTT assay and calculated on Graph Pad Prism software. The IC₅₀ values for the cell lines varied from 0.12 to 2.60% v/v. The maximum tolerated concentrations (MTC) ranged between 0.03 and 1.17 % v/v. The MICs covered a wide range between 3.125 and more than 25 % v/v. Ethanol (EtOH) was not cytotoxic for either cell lines up to 2 % v/v or microorganisms up to 25 % v/v except CCL-1 cells (MTC = 1.15% v/v). 2-Methoxyethanol (MEtOH) and polyethylene glycol (PEG-400) exhibited low cytotoxicity in cell lines (MTC = 1-2 % v/v) in contrast to dimethyl sulfoxide, dimethoxyethane and dimethylformamide. Tumorigenic cell lines were more tolerable than non-tumorigenic. EtOH, MeOH and PEG-400 were most suitable for antimicrobial susceptibility testing (MICs ≥ 25 % v/v). The data obtained can be useful for *in vitro* estimation of the cytotoxic effects of biologically active molecules in eukaryotic and prokaryotic cells.

Keywords: *cytotoxicity, antimicrobial capacity, organic solvents, plant extracts.*

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This study was supported by the National Scientific Program “Health foods for strong bioeconomics and quality of life” (2018 – 2021).

SESSION 2: Natural health products

Lecture 1. ANTITUMOR ACTIVITY OF BIOACTIVE COMPOUNDS FROM MARINE SNAIL *RAPANA VENOSA* AND GARDEN SNAIL *HELIX ASPERSA* AGAINST A PANEL OF HUMAN BREAST CANCER CELL LINES

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Hemolymph from marine snail *Rapana venosa* (living in the Black sea) and the mucus from garden snail *Helix aspersa*, are rich in various bioactive substances with potential application in medicine. Breast cancer is the most common malignancy among women. In this study we tested different fractions from *H. aspersa* and *R. venosa* for antitumor activity against breast cancer cellular models. We found that low concentrations of two mucus fractions from *H. aspersa* (Mw above 20 kDa and above 50 kDa), and one (Mw between 50 and 100 kDa) from marine snail *R. venosa* reduced cell viability in both MCF-7 and MDA-MB-231 breast cancer cell lines. Interestingly lower toxicity was observed in MCF-10A cells which are commonly used as a model of normal breast cells. Next we combine classical chemotherapy drugs cis-Platin and Tamoxifen with the active fractions from the hemolymph of *R. venosa* and the mucus of *H. aspersa* to look if any synergistic effect of action against the tumor cell lines appear. Indeed, we found synergistic anti-proliferative effect most pronounced when using the active fraction between 50-100 kDa from hemolymph of *R. venosa* in combination with cis-Pt for the treatment of MDA-MB-231 (triple negative) cell line. Even more the microscopic observations on MDA-MB-231 cells showed substantially disturbed morphology after treatment with the fraction from structural subunit RvH1 in combination with cis-Platin in comparison to non-treated cells or treated with RvH1 alone.

These results may be considered as promising beginning and good base for further investigations of antitumor activity of novel bioactive compounds from hemolymph of marine snail *R. venosa* and garden snail *H. aspersa*.

Keywords: *Hemolymph, Rapana venosa, Helix aspersa, breast cancer, antitumor activity*

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Lecture 2. THE EFFECT OF A NEW SYNTETIC FERROCENE CONTAINING CAMPHOR SULFONAMIDE ON BREAST CANCER CELL LINES

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With the increasing number of cancer patients, the need of new anti-cancer drugs is becoming increasingly urgent, and the requirements for these get more complex. Although chemotherapy is the gold standard for cancer treatment the use of the existing chemotherapeutic agents is often limited due to negative side effects caused by the lack of selectivity. Chemotherapeutic agents, combining

several functional groups in one molecule, can modulate different regulatory pathways in the cell and reach higher efficacy compared to drugs affecting only one cellular process. The purpose of this study was to characterize the molecular mechanisms of cytotoxicity of new synthesized ferrocene-containing camphor sulfonamide 1-((1S,4S)-3-((E)-ferrocenylmethylidene)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)-N-(tert-butyl) methanesulfonamide (DK-164) in MDA-MB231, MCF-7 breast cancer cell lines and in H1299 and A549 lung cancer cells lines, as well as in noncancerous cells MCF-10 and MRC5.

The results of our MTT-dye reduction and clonogenic assays indicate that the compound has great antitumor activity and the cytotoxic effect is more pronounced in cancer than in noncancerous cells. The apoptotic and autophagic effects caused by DK-164 were evaluated by annexin v binding and LC3 puncta formation assays respectively. Using flow cytometry, we could show, that IC₅₀ of the substance caused a cell cycle arrest in G1 phase and induced apoptosis up to 24 hours in all tumor cells. In all cases the cancer cells MCF-7 and A549, which has a functional p53 protein, were stronger affected. To reveal the molecular mechanism of action and to identify clues for the observed selectivity of the compound, we visualized the dynamics of different key proteins such as p53, NFκB, LC3, beclin and vimentin using Western blot and immunofluorescent techniques.

To overcome the problems resulting from the poor solubility of the ferrocene compound in aqueous media and to get use of the benefits of the polymeric drug delivery systems, two novel micellar carriers were loaded with DK-164. The biological activity of the newly constructed polymeric system was confirmed by a series of experiments.

Keywords: *ferrocene, chemotherapy, breast cancer, lung cancer, apoptosis, autophagy, cytotoxicity, polymer system*

ACKNOWLEDGMENTS

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Lecture 3. EFFECT OF CANNABIDIOL ON A PANEL OF LUNG CANCER

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Cannabidiol is one of the 113 cannabinoids extracted from cannabis plants and is characterized with complex and contradictory biological function. Through the CB-2 receptors cannabidiol also affects the immune system. There are intensive studies on its antitumor effects.

The aim of the study is to evaluate the cytotoxic activity of cannabidiol and its effect on proteins regulating autophagy and apoptosis. We used human lung cancer cell lines and lung non-cancer cell line as a control, with a different profile and degree of expression of oncogenes in *in vitro* cell-based assays.

In this study were used human cancer cell lines - two lung cell lines A549 (p53 positive) and H1299 (p53 negative) and non-cancer lung fibroblast cell line – MRC-5. A standard protocol for MTT assay was used to determine the cytotoxic effect of CBD on the cell lines described above. Analysis of the dose-response curve was done using the Software GraphPad PRISM. The IC-50 for H1299, A549 and MRC-5 were 22,87 μM, 16.4 μM and 18.5 μM respectively.

An optimised protocol for immunofluorescence was used to visualise localization and expression of key cellular proteins like Beclin, LC3, Caspase 3/7 and HMGB1 that determined the cellular response after the treatment. Etoposide was used as positive control. Imaging was done with Zeiss Axiovert 200M and the images were analysed by ImageJ software. The processed immunofluorescent images showed induction of caspase 3/7 expression in A549 cells treated with cannabidiol (10 μM) with lesser expression of the autophagy markers LC3 and Beclin compared to the p53^{-/-} H1299 where the treatment induced autophagy. In both lung cancer cell lines colocalization of HMGB1 and Beclin was observed. There was greater quantity of this protein complex in H1299 and it was mainly

localized in the cytoplasm. The expression of LC3 after treatment with cannabidiol was enhanced in both lung cancer cell lines, but yet again it was more profound in H1299. The MRC-5 and A549 cells showed similar pattern of their protein expression. Apoptosis was determined by Flow cytometry with Annexin V and PI staining. The treatment with three different concentrations of cannabidiol (IC25, IC50, IC75) has shown an increase in the population of apoptotic cells in each of the tested cell lines.

Keywords: *cannabidiol, apoptosis, autophagy, lung cancer, in vitro cell based assays*

ACKNOWLEDGEMENTS

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Lecture 4. THE STUDY OF THE S-PHASE OF THE CELL CYCLE - MOTIVATION AND METHODS

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During the S-phase of the cell cycle the genome needs to be fully and faithfully replicated. Replication fork slowing or stalling is a condition indicated as replication stress, which could result in DNA damage, cell death or oncogenic transformation. Tumour cells proliferate rapidly leading to higher levels of replication stress and consequently DNA damage, driving tumourigenesis. At the same time the mechanisms that protect from replication stress are viewed as promising targets to selectively eliminate cancer cells by bringing their already high replication stress to levels incompatible with life. Therefore, compounds, including from natural sources, could be effective in cancer treatment if they could interfere with the mechanisms protecting from replication stress. The aim of this report is to present the key methods to study replication and replication stress response in populations of cultured cells, as well as at the level of single replication forks. These methods are illustrated by their application to study the effects of natural compounds on replication. Such studies should help find compounds that could sensitize cells to replication stress and potentially able to augment existing therapeutic approaches.

Keywords: *S-phase, DNA replication, DNA replication stress, natural compounds*

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Lecture 5. NATURAL MEDICINE: THE SEARCH FOR POTENTIAL ANTITUMOR PROPERTIES AMONG THE MUSHROOMS OF BULGARIA

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Bulgaria's wild-growing mushrooms have, until recently, largely gone unexplored by the scientific community. While many of the native mushrooms of the region have long been prized by residents as a valuable food source, they have generally not been thought of as having the medicinal value of certain herbs, for example, perhaps due to the lack of a well-established place for mushrooms in the medicinal folk tradition of Bulgaria. However, the growing body of scientific literature confirming the diverse beneficial effects of many Asian mushrooms (long held in high regard by ancient medicinal traditions of their respective regions), as well as the somewhat surprising discovery that some of these same mushrooms can be found in Bulgaria, has sparked our interest in exploring the effects they may have on different types of cancer, with the goal of either complementing existing

treatments or, perhaps, uncovering new treatments based on compounds isolated from mushroom extracts.

In this work we evaluated the primary antitumor activity of different water and ethanol extracts, prepared in our laboratory, from five species of mushroom obtained from the Vitosha nature reserve near Sofia – *Trametes versicolor*, *Lenzites betulina*, *Fomes fomentarius*, *Piptoporus betulinus* and *Fomitopsis betulina* – as well as the activity of an ethanol extract prepared from *A. muscaria* which has been kindly provided to us by Vladimir Vazharov.

The cell lines used in our study include MRC-5 fibroblasts, H1299 non-small lung carcinoma cells, A549 adenocarcinomic alveolar basal epithelial cells, MDA-MB231 mammary adenocarcinoma cells and a panel of skin cancer-derived cell lines - A375, A375 KRAS, Hs 895 and Hs 895.T. We performed a MTT assay using a standard protocol and the data was analyzed using GraphPad Prism™ software. Our results show varying degrees of primary cytotoxic activity; the half-maximal inhibitory concentration (IC₅₀) was established for, and compared between, all the species of mushroom, as well as between the different types of extract. In addition, we discovered that some of the ethanol extracts have a higher IC₅₀ than pure ethanol, which might point to a potential use as a protective agent to be used in conjunction with chemotherapy. We believe the results to be promising and, going forward, hope to establish the effects of the extracts and their metabolites on different cancers in model organisms.

Keywords: mushrooms, cancer, cytotoxicity, natural medicine

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Lecture 6. MEDICINAL MUSHROOMS OF BULGARIA

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Mushrooms, with their incredible colors and curious shapes, have attracted man's attention since time immemorial. The unique taste and texture of those mushrooms that have been discovered to be edible have also made them into a staple delicacy in many parts of the world. Apart from being a tasty addition to our tables, however, these unique organisms can provide a plethora of medicinal benefits, many of which have as of yet gone without much publicity in the Western world, in spite of them being discovered in prehistoric times - as evidenced, for example, by the presence of certain species of medicinal mushrooms in the pouch of a 5200-year-old mummy (affectionately nicknamed Ötzi) found frozen in the Ötztal Alps in 1992.

Bulgaria is a country rich in high-quality wild-growing medicinal mushrooms. Our mountains are home to many species, such as the well-known reishi (*Ganoderma lucidum*), turkey's tail (*Trametes versicolor*), chaga (*Inonotus obliquus*) and others, that have been used medicinally in many parts of the world and have been the subject of scientific inquiry. There are many other species, however, which have not enjoyed much popularity with the general public and whose incredible healing potential should most definitely not be overlooked. In this talk Vladimir Vazharov, who has spent a lifetime documenting the benefits of many different mushroom species – from the well-known to the relatively obscure – and is the author of the book 'Medicinal mushrooms of Bulgaria', will introduce us to the hidden world of medicinal mushrooms, their use, and their habitat.

Keywords: mushrooms, natural medicine

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Lecture 7. MOLECULAR MODELLING OF ANTIMICROBIAL PEPTIDE MIXTURES FROM THE MUCUS OF GARDEN SNAIL

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Antimicrobial peptides are a key element in the primary host defense against microbial invasions in all eukaryotes. They exhibit rapid and efficient antimicrobial activity against a range of pathogens – Gram-positive and Gram-negative bacteria, fungi, parasites, and some viruses. Despite the millions of years of co-evolution, bacteria have failed so far to develop even limited resistance against their action. All this makes AMPs a promising therapeutic alternative to the conventional antibiotics in the face of dramatically increasing bacterial (multi-drug) resistance.

AMPs come in nature in the form of multicomponent secretory fluids that exhibit certain biological activity. Their antimicrobial action is not completely understood; neither is their behaviour in bodily liquids prior to attacking the target membrane. *In silico* experiments are designed to speed-up the identification of the active components in these substances, understanding of their structural specifics and biodynamics. By means of molecular dynamics simulations we develop 3D models of some newly isolated at IOCCP-BAS peptides from the mucus of the garden snail *Cornu Aspersa*, study their solvation behavior and show that particularly extended AMPs tend to self-associate in clusters and this process also drives their convergence into the biological fold.

Keywords: *computational modelling, 3D structure, solvation behavior, self-association*

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Lecture 8. STUDYING THE ACTIVITY OF BIOACTIVE COMPOUNDS ISOLATED FROM GARDEN SNAILS (*HELIX ASPERSA*, *HELIX LUCORUM*) AND MURINE SNAILS (*RAPANA VENOSA*) AGAINST VARIOUS HUMAN CANCER CELL LINES

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Progress in biomedicine over the last ten years has led to significant changes in anti-tumor therapy. With the identification of new target molecules and processes, the focus of therapeutic approaches is shifting from broad spectrum cytotoxic agents to more specific analogues and/or methods tailored to the type of cancer and pathological context of the patient - to the search for personalized approaches. The persistent emergence of resistant and multiresistant tumors clearly emphasizes the need of developing alternative low-toxicity natural remedies for more effective treatment.

The aim of the study is to evaluate the cytotoxic activity of natural products with low toxicity isolated from snails (*Helix aspersa*, *Helix lucorum*) and *Rapana* (*Rapana venosa*) - native hemocyanins, hemocyanin subunits, hydrolysates, mucus and hemolymph. The hemocyanins (Hcs) are extracellular copper proteins isolated from different arthropod and mollusc species. Hcs are oxygen transporter molecules and normally are freely dissolved in the hemolymph of these animals.

Using various human cancer cell lines, with a different profile and degree of expression of oncogenes and/or drug resistance genes, we conducted an *in vitro* cell-based assay in which the cells were

treated with Hcs, mucus and hemolymph. Half-maximal inhibitory concentration (IC50) of the bioactive compounds was obtained from an experimentally derived dose-response curve. We also tested combinations of the products with cannabidiol (CBD) and cisplatin (cis-Pt) for synergistic effect.

Two of the hemocyanins showed anti-tumor activity alone, while three others showed promising synergistic effect in combination with CBD. Hemolymph from *R. venosa* with molecular weight (Mw) 50-100 kDa exhibited high activity alone and in combinations with CBD and cisplatin.

Keywords: *hemocyanins, Helix aspersa, Helix lucorum, Rapana venosa, mucus, hemolymph, anti-tumor activity*

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Lecture 9. EFFECTS OF INFLUENCE OF PEPTIDE FRACTIONS FROM MUCUS OF CORNU ASPERSUM AGAINST ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS И CLOSTRIDIUM PERFRINGENS

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The pathogens *Escherichia coli* NBIMCC 8785, *Staphylococcus aureus*, *Clostridium perfringens* NBIMCC 8615 are the most dispreads bacteria, provoked infections of different human organs. In the last years because of the increase of resistance of the bacteria against antibiotics, the alternative agents with antimicrobial actions have been searched and investigated. One of the variants is the application of different natural substances, especially the peptides with antimicrobial effect. Natural products have long played a major role in medicine and science. The garden snail *Cornu aspersa* is a rich source of biologically active natural substances which might be an important source for new drugs to treat human disease.

The purpose of the study was the investigation of the effect of peptide fractions against *Escherichia coli* NBIMCC 8785, *Staphylococcus aureus*, *Clostridium perfringens* NBIMCC 8615. Seven fractions containing compounds with Mw <3 kDa; <10 kDa; <20 kDa; >20 kDa; and between 3-5 kDa; 5-10 kDa; and 10-30 kDa, isolated from mucus of snail *Cornu aspersum*, were investigated. The peptide effect has been analyzed by means of method of migration of antibacterial factor from hole in agar. In parallel the morphological changes of the bacterial cells and their complexes by means of scan electronic microscope and confocal fluorescence microscope have been studied. The results showed that different peptide fractions had various strong antibacterial effect against the investigated pathogens, measured in mm²/mgPr./mkL. The SEM analysis confirmed the antibacterial effect and illustrated the strong deformation of the bacterial cells influenced by peptide fractions. The craters, vesicles on the surface of the bacteria have been ascertained by SEM.

These results revealed that the peptide fractions with Mw 10 – 20 kDa is effective against *E. coli*; and the protein fraction >30 kDa against the bacterial strain *C. perfringens*. The results are perspective for public health in the direction to “live close to the nature with the principles of green life”.

Keywords: *Cornu aspersa* mucus; peptides; antibacterial activity; bacterial strains *Escherichia coli* NBIMCC 8785; *Staphylococcus aureus* and *Clostridium perfringens* NBIMCC 8615

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Lecture 10. ANTIMICROBIAL ACTIVITY OF OREGANO OIL ENCAPSULATED IN CHITOSAN-ALGINATE NANOPARTICLES

Prof. Dr. Krassimira Yoncheva¹, Assoc. Prof. Dr. Niko Benbassat¹, Assist. Prof. Dr. Maya M. Zaharieva², Assist. Prof. Dr. Lyudmila Dimitrova², Assoc. Prof. Dr. Alexander Kroumov², Assoc. Prof. Dr. Ivanka Spassova³, Prof. Dr. Daniela Kovacheva³, Prof. DSc. Hristo M. Najdenski²

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Aim of the study is to load oregano oil in delivery systems based on hydrophilic vehicles in order to expand its therapeutic impact in the treatment of bacterial and fungal infections. Nanosystems with oregano oil can be used successfully also in the food industry for preservation of meat products. The encapsulation of the oregano oil into chitosan-alginate nanoparticles was performed by emulsification and consequent electrostatic gelation of both biopolymers. Physicochemical characterization of the oil loaded chitosan-alginate nanoparticles included determination of their size, FTIR spectroscopy, thermogravimetric and XRD analyses. The microbicidal effect and the bacterial respiratory activity were determined by ISO 20776-1:2006(E) and MTT tests, respectively. Seven pathogenic bacterial and one fungal strain were evaluated. The data were analysed with the GraphPad Prism software. The physicochemical characterization showed that the oil loaded nanoparticles had small size and negative charge. The data from FTIR spectroscopy and XRD analyses revealed successful encapsulation of the oil into the polymer nanoparticles. The results from thermogravimetry suggested an improved thermal stability of the encapsulated oil. The oregano oil exhibited strong antimicrobial activity. The MICs of the encapsulated oil were up to ten fold lower than that of the pure oil. The polymer nanoparticles inhibited the respiratory activity of the bacterial strains to a lower extent than the pure oil but the bacterial growth inhibition occurred at significantly lower concentrations than those of the pure oil. The encapsulated oregano oil possesses strong antimicrobial activity and is promising for development as a food additive.

Keywords: *oregano oil, biopolymers, antimicrobial activity*

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Lecture 11. EHRLICH ASCITES CARCINOMA - A MOUSE MODEL OF BREAST CANCER

Inna Sulikovska¹, Assoc. Prof. Dr. Ivan Iliev¹, Assist. Prof. Zdravka Petrova¹, Chief Assist. Prof. Dr. Katerina Todorova¹, Chief Assist. Prof. Dr. Ani Georgieva¹

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Ehrlich ascites carcinoma is rapidly growing mouse breast adenocarcinoma. The advantage of this tumor experimental model of murine mammary cancer that its cells can be carried in outbred mice by

serial intraperitoneal passages and that it has similar sensitive to chemotherapy as in human breast cancer.

The aim of present study was to characterize the Ehrlich ascites carcinoma cell line (EAC), which will serve as an experimental model for testing substances with antitumor activity.

Tumor cells isolated from the ascites forms derived from peritoneal cavity. The morphological heterogeneity of cells was studied by microscopic methods. Receptor status of progesterone, estrogen and Her-2 was determine by monoclonal antibody. The antiproliferative activity of standard cytostatics that we used for treatment of breast cancer was analyzed by Neutral Red Uptake Assay.

The cytological analysis has shown many apoptotic cells with pyknotic nuclei and condensation of the cytoplasm. That is correlate with double fluorochrome results. The presence of progesteron, estrogen and Her-2 receptor is not detecting by antibody, which confirms aggressive status of tumor. The IC₅₀ values for cytostatics and they toxic effects will be serve as a positive control for subsequent studies.

Keywords: *Ehrlich Ascites Carcinoma, cytostatic, morphology, receptor status*

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Lecture 12. ASSESSMENT OF THE CYTOTOXIC EFFECTS AND ANTIPROLIFERATIVE ACTIVITY OF HEMOCYANINS FROM *HELIX LUCORUM*, *HELIX ASPERSA* AND *RAPANA VENOSA*

BSc. Elena Ivanova¹, Assoc. Prof. Dr. Ivan Iliev¹, MSc. Inna Sulikovska, BSc.Dardana Manga¹, Chief Assist. Prof. Dr. Katerina Todorova¹, Chief Assist. Prof. Dr. Ani Georgieva¹, Chief Assist. Prof. Dr. Valeriya Dilcheva¹, Chief Assist. Prof. Dr. Ivelin Vladov¹, Prof. Dr. Svetlozara Petkova¹, Prof. Dr. Reneta Toshkova¹ and Prof. Dr. Pavlina Dolashka²

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Hemocyanins are copper-containing respiratory glycoproteins with quaternary structure, localized in the haemolymph of some invertebrate gastropods belonging to Molluska and Arthropod families. They are often used as carrier proteins in antibody production and direct application in bladder cancer. There is data mainly about Keyhole Lympet Haemocyanin, isolated from the marine snail *Megatura crenulata*. However, the restricted geographical areal of *M. crenulata* does not allow the extraction of huge quantity hemocyanin.

As an alternative of KLH we propose haemocyanins, isolated from *Helix lucorum*, *Helix aspersa* and *Rapana venosa*, which are widespread, including Bulgaria.

The aim of the present study was to determine the citotoxicity and antiproliferative activity of hemocyanins isolated from *Rapana venosa*, *Helix lucorum* and *Helix aspersa*. The biological activity of the hemocyanins were studied under *in vitro* and *in vivo* conditions. For *In vitro* safety testing were used 3T3 Neutral Red Uptake Cytotoxicity test. Experiments for antiproliferative activity were performed on a panel of cell lines - a model of breast cancer. The effects of the hemocyanins on the cell viability were analyzed by MTT Dye Reduction assay. Ehrlich Ascites Carcinoma was used as a model of tumor disease *in vivo*.

Evaluation of *in vitro* cytotoxicity shows that the tested hemocyanins are non-toxic. In the *in vivo* experiments, the results of histochemical analysis, examining organ and systemic toxicity observed that there were no acute, subacute or chronic toxic effects in the treated animals.

The high antitumor activity of α -HaH (IC₅₀ = 258,9 μ g/ml for MCF-7 cell line) determined under *in vitro* conditions was also observed in *in vivo* experiments for survival of tumor-bearing animals.

Keywords: *hemocyanins, cytotoxicity, antiproliferative activity*

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Lecture 13. EXCELLENCE IN ROUTINE AND SCIENCE, LKB

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LKB has been present on the Bulgarian market since 1975. It was founded as a representative office of LKB-Sweden. The company offers products in the field of laboratory, pilot and industrial biotechnology, as well as in the field of particle characterization.

LKB is an authorized representative of Cytiva, USA based in Sweden, Germany, the United Kingdom and the United States. In its current state, the company is the result of the successive merger and/ or transformation of several companies such as LKB, Pharmacia Biotech, Amersham, General Electric and others. The portfolio covers apparatus, systems and reagents for purification and characterization of proteins and other biomolecules used in routine laboratory and production practices as well as for special purposes. Areas of application are biotechnology, biochemistry where the most commonly used methods are chromatography, 1-D and 2-D electrophoresis and spectrophotometry of biomolecules, PCR, etc. The company offers automatic, semi-automatic and manual systems for the above methods with different capacities and customized specifications depending on the specific customer applications. Most of the products, and especially the reagents, are subject to company patents (eg chromatographic media) and the devices have specifications that meet the requirements of GLP and the pharmacopoeia.

LKB is well known to those working in the field of biotechnology on a laboratory scale, as well as to those who deal with their application for the needs of production. LKB has a qualified staff that performs marketing, service and post-installation maintenance.

SESSION 3: Ecological products and processes

Lecture 1. PROTEOMIC ANALYSIS OF INTRACELLULAR EXTRACTS OF *SACCHAROMYCES CEREVISIAE* TREATED WITH TOXINS IN BOTH LOG AND STATIONARY PHASE

Assist. Asya Daskalova¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Assoc. Prof. Dr. Ventseslava Petrova², Prof. Wolfgang Voelter³, Prof. DSc. Pavlina Dolashka¹

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Thanks to the determination of the genome sequence of the *Saccharomyces cerevisiae*, nowadays, it is widely spread to use *S. cerevisiae* as a model organism for biochemical and molecular researches. The aim of that research is to determine proteins responsible for the adaptation to oxidative and toxic stress in Log and stationary phase. Therefore, the yeast strain *S. cerevisiae* BY4741, was treated with oxidative and drug agents (H₂O₂, menadion, zeocyn and ibuprofen) in both phases. Proteomic analysis, including SDS PAGE coupled with mass spectrometry was used to explain that mechanism. In order to identify the proteins the analysis of the results from MALDI-TOF/MS spectra, Mascot database-Fingerprint was used to identify the proteomic analysis. Seven bands were found and made sense and corresponding proteins were proposed: Cytochrome c peroxidase, Glutathione S-Transferase Omega-like, NAPDH-dependent diflavin reductase, DNA replication fork-blocking protein, Putative aryl alcohol dehydrogenase, AP-1-like transcription factor YAP5, GTP-binding protein. A deeper investigation of the conserved mechanisms expressing entry into, survival in and exit from quiescence in higher eukaryotes will help the development of new anticancer therapies, the process of aging and neurodegenerative diseases.

Keywords: *oxidative stress, Saccharomyces cerevisiae, SDS PAGE, MALDI- TOF MS, proteins*

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Lecture 2. METABOLIC PROFILE OF BIOACTIVE COMPONENTS FROM *GENTIANA* SPECIES

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Genus *Gentiana* L. is distributed in Europe, Asia, North and South America and comprises over 300 species, 11 of them are distributed in the mountainous part of Bulgaria. *Gentiana* species have been used as medical plants for centuries due to the presence of bitter secoiridoid glycosides. These secoiridoid glycosides show interesting biological activities, such as antibacterial, antifungal, anti-inflammatory, antitumor, gastroprotective and hepatoprotective.

The objective of this study was phytochemical investigation of the iridoid composition of the *Gentiana cruciata* (commercial sample and Slavyanka origin) and *Gentiana asclepiadea* (Rhodope and Vitosha origin) and determination the similarities and differences in the content of the main biologically active metabolites. The carried out investigation afforded a total of 7 iridoid glycosides: gentiopicroside, swertiamarine, depressine, trifloroside, loganic acid, eustomoside and

eustomorusside. The isolated individual compounds were used to perform chemical profiling of *Gentiana* extracts obtained by NMR spectroscopy. The results showed a difference in the iridoid composition, both between species and intraspecific, depending on the origin of the sample. *G. cruciata* (commercial sample) and *G. asclepiadea* (Vitoshka) showed a similar metabolic profile, including gentiopicroside as main component, while in *G. cruciata* (Slavyanka) and *G. asclepiadea* (Rhodope) the main component is eustomoside.

The iridoid profile of *Gentiana cruciata* and *Gentiana asclepiadea* growing in different Bulgaria origin was investigated and seven iridoid glycosides were determined. The structures of the isolated compounds were determined by 1D and 2D NMR experiments. Secoiridoid glycosides eustomoside and eustomorusside were reported the first time for genus *Gentiana*. NMR-based metabolomics approach has been applied to study metabolic differentiations of the species. The results showed a difference in the iridoid composition, both between species and intraspecific, depending on the origin of the sample.

Keywords: *Gentiana*, iridoid glycosides, metabolic differentiations

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Lecture 3. INVESTIGATION OF DIFFERENT FRACTIONS FROM MUCUS OF THE GARDEN SNAIL *CORNU ASPERSUM* WITH ANTIBACTERIAL ACTIVITIES

Assoc. Prof. Dr. Lyudmila Velkova¹, Assoc. Prof. Dr. Aleksandar Dolashki¹, Elmira Daskalova², N. Zheleva², Prof. DSc. Yana Topalova², Asist. Ventseslav Atanasov¹, Prof. Wolfgang Voelter³ and Prof. DSc. Pavlina Dolashka¹

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Natural sources may lead to basic research on potential bioactive components for various drug discovery. The garden snail *Cornu aspersum* is a rich source of biologically active natural substances that might be an important source for new drugs to treat different human disease. Based on our previous studies, nine fractions containing compounds with Mw <3 kDa; <10 kDa; <20 kDa; >20 kDa; >30 kDa>50 kDa and between 3 and 5 kDa; 5 and 10 kDa; and 10 and 30 kDa were purified from the mucus of *C. aspersum* and analyzed by tandem mass spectrometry (MALDI-TOF/TOF). Twenty novel peptides with potential antibacterial activity were identified by *de novo* MS/MS sequencing using tandem mass spectrometry. The different fractions were tested for antibacterial activity against Gram⁻ (*Pseudomonas aureofaciens* and *Escherichia coli*) and Gram⁺ (*Brevibacillus laterosporus*) bacterial strains as well the anaerobic bacterium *Clostridium perfringens*. These results revealed that the peptide fractions exhibit a predominant antibacterial activity against *B. laterosporus*; the fraction with Mw 10–30 kDa against *E. coli*; another peptide fraction <20 kDa against *P. aureofaciens*; and the protein fraction >20 kDa against the bacterial strain *C. perfringens*. The discovery of new antimicrobial peptides (AMPs) from natural sources is of great importance for public health due to the AMPs effective antimicrobial activities and low resistance rates.

Keywords: *Cornu aspersum* mucus, antimicrobial peptides, antibacterial activity

ACKNOWLEDGEMENTS

This work was supported by the contract No. DN 01/14 of 19.12.16., funded by the Scientific Research Fund of the Ministry of Education and Science, Bulgaria and Project VS.076.18N(FWO) funded by Bulgaria and Belgium

Lecture 4. INVESTIGATION OF ANTITUMOR POTENTIAL OF HEMOCYANINS FROM GASTROPODAN AGAINST T24 BLADDER CARCINOMA

Dimitar Kaynarov¹, Assoc. Prof. Dr. Aleksandar Dolashki¹, Assist. Prof. Dr. Olga Boyanova², Assoc. Prof. Dr. Lyudmila Velkova¹, Prof. Wolfgang Voelter³ and Prof. DSc. Pavlina Dolashka¹

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Hemocyanins (Hcs) are copper-containing respiratory proteins in hemolymph of many molluscs and arthropods. Most molluscan Hcs are glycoproteins with high molecular masses and complex quaternary and oligosaccharide structures. Some of them possess significant immunological and antitumour potential, enabling their application in oncology.

The antitumour activity of Hcs from marine snails *Rapana venosa* (RvH), giant keyhole limpet *Megathura crenulata* (KLH), and garden snails *Helix lucorum* (HIH), and their different forms were investigated *in vitro* on bladder cancer permanent cell line T24 and the normal urothelial cell line HL 10/29 in comparison to doxorubicin. The obtained results showed that the T24 tumour cell line is sensitive to the action of the tested isoforms and glycosylated FUs of these hemocyanins. A dose- and time-dependent selective growth inhibition of T24 cells was established after incubation with isoforms β c-HIH and RvHII and FUs β c-HIH-h and RvHII-e. Furthermore, the fluorescent micrographs showed no apoptotic or necrotic alterations of the normal urothelial cell line HL 10/29. The FU β c-HIH-h demonstrated highest antiproliferative effect (similarly to the doxorubicin) in which a primarily apoptotic and less necrotic alterations in the tumour cells were observed. Using proteome analysis seven down-regulated and three up-regulated proteins were identified, which may be associated with the apoptosis pathway.

The present study is the first report of protein expression in T24 human bladder carcinoma cells under the influence of FU β c-HIH-h which is probably due to the specific oligosaccharide structures, rich in methylated hexoses. This might be essential for the interactions of β c-HIH-h with carbohydrate residues exposed on the surface of tumour cells and the observed antitumour activity.

Keywords: hemocyanins (Hcs), garden snails *Helix lucorum*, bladder cancer permanent cell line T24, antiproliferative effect.

ACKNOWLEDGEMENTS

This work was supported by the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)” approved by DCM № 658/14.09.2018 funded by the Bulgarian Ministry of Education and Science, Bulgaria

Lecture 5. CORTEX AND HIPPOCAMP PROTEIN PROFILE ON SCOPOLAMINE RAT MODEL OF ALZHEIMER’S DISEASE WITH APPLICATION OF SNAIL EXTRACT FROM *HELIX ASPERSA* AS NEUROPROTECTIVE AGENT

Assist. Ventseslav Atanasov¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Prof. Dr. Lubka Tancheva², Assoc. Prof. Dr. Aleksandar Dolashki¹, Prof. Dr. Reni Kalfin², Prof. DSc. Pavlina Dolashka¹

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Alzheimer’s disease (AD) is the most widespread neurodegenerative disorder. The scopolamine is frequently used agent for induction of Alzheimer in experimental animals. We used scopolamine model for assessment of potential neuroprotective effect of extract from garden snail *Helix aspersa* on neurodegenerative processes *in vivo*. Male sexually mature experimental rats were used, divided on three groups: a control group of healthy rats, a scopolamine group (treated with scopolamine) and an experimental group treated with scopolamine and snail extract together. Two major memory-

related brain structures (hippocampus and prefrontal cortex) are isolated. The obtained proteins were separated by SDS – PAGE and analyzed with MALDI-MS. Using MASCOT Peptide Mass Fingerprint the cortex and hippocampal proteins have been identified and compared.

Keywords: *Alzheimer's disease (AD), scopolamine, snail extract, neuroprotective effect, rat brain*

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This work was supported by the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM № 658/14.09.2018 funded by the Bulgarian Ministry of Education and Science, Bulgaria

Lecture 6. A NEW APPROACH IN THE TREATMENT OF DIFFICULT TO HEAL AND CHRONIC WOUNDS

Dr. Momchil Kermedchiev^{1,2}, Dr. Irina Michael¹, Dr. Evgenia Peneva¹, Dr. Radka Lazarova¹, Dr. Stela Jordanova¹, Prof. DSc. Pavlina Dolashka²

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Some wounds do not heal normally and present a challenge. They can be considered difficult to heal and chronic wounds. There are many reasons for such wounds and when they occur, the physician must take into account all factors related to wound. We need to take appropriate and timely measures to correct the underlying cause for difficult to heal wound and it should be treated with a new methodology.

Knowledge of the therapeutic action of mucus from snails and various kinds of medicinal herbs in the treatment of wounds have been known since the time of the known written story reached us. Snail farms and their use in the treatment of wounds are described in Egyptian cryptograms and papyri, Pliny the Elder (23 AD) writes that snails are "a sovereign means of treating pain associated with burns, abscesses and other wounds".

Patients with difficult to heal and chronic wounds of different age and genesis (Diabetic gangrene, Chronic venous insufficiency, Chronic arterial insufficiency and pressure ulcers), as well as with different comorbidities, were selected prospectively of different sexes and ages.

Applied is the same prospective new protocol, new wound antisepsis approach, new pace and type of dressings, treatment plan, training the patient and kinsfolk to self-help and dressings.

Results of a number of parameters were reported during the treatment process: Local wound status, pH-metry, local inflammatory process, bacterial flora, pain level, healing rate.

The new approach used in the treatment of difficult to heal wounds with snail mucus from *Helix Aspersa* and herbal essential oils shows promising results.

There is a significant reduction in the time for wound healing, rapid and effective reduction of the local inflammatory process and chronic pain. Improving the general and psycho-emotional state of the patient, significantly improving the quality of life. The obtained results suggest that the healing of such wounds is promoted not only by limiting bacterial infection, but also by stimulating the growth of tissues and providing them with an adequate local alkaline-acid, moist and nutritious environment. Early and regular application of our proposed new approach to treatment will lead to the prevention of amputation of the legs and would serve as a basic therapy for the treatment of chronic wounds.

Keywords: *Hard to heal wounds, chronic wounds, treatment, snail Helix Aspersa, Essential oils.*

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Lecture 7. FRESH EXTRACT OF SNAIL (HELIX ASPERSA) IMPROVES MEMORY CAPACITY IN EXPERIMENTAL MODEL OF DEMENTIA IN RATS

Prof. Dr. Lyubka Tancheva¹, Assist. Prof. Maria Lazarova¹, Prof. DSc. Pavlina Dolashka², Assoc. Prof. Dr. Alexander Dolashki², Assoc. Prof. Dr. Lyudmila Velkova², Assist. Miroslava Stefanova, Assist. Diamara Uzunova, Petya GavriloVA, Assoc. Prof. Dr. Albena Alexandrova^{1,3}, Assist. Prof. Elina Tsvetanova¹, PhD student Yordan HodzeV¹, Prof. Dr. Reni Kalfin¹

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The emblematic for our days Alzheimer's diseases (AD) is called "the epidemic of 21st century". More researchers believe in complex mechanism of AD, hence multi-target treatment with natural products to be more effective. There are many data about preventive and therapeutic effect of Snail extract against some diseases because of its rich biological activities (antioxidant, anti-inflammatory, membrane stabilizing etc.). But there are sparse data in the literature about its effect on the neurodegenerative disorders. The aim of the current study is to clarify some main mechanisms involved in memory improving effect of fresh mucus snail extract on experimental model of dementia in rats produced by Scopolamine treatment (2 mg/kg, ip, 11 days). We found that snail extract significantly compensated the memory deficits, observed in dement rats, and has a positive effect on short- and long- term memory processes. The learning and memory capacity in dement rats were significantly improved and almost reached levels of healthy controls. Memory protective effect of snail extract was accompanied by significant inhibition of acetylcholine esterase activity in the hippocampus, but not in the cortex. Snail extract exerted neuromodulatory activity - it increased the content of dopamine and noradrenaline in the cortex as well as in the hippocampus, and totally reversed the effect of scopolamine. Oxidative damages observed in brains of dement rats were significantly corrected by snail extract administration. Lipid peroxidation, total glutathione, activities of enzymes catalase and glutathione peroxidase assessed in brain structures related to the memory process were normalized after snail extract treatment. Cluster model of dementia and correlation analysis performed evaluated the correlations among parameters studied.

Keywords: *Snail extract, Dementia, Memory, Alzheimer's disease*

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This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018) under the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM # 658/14.09.2018.

Lecture 8. VIRUCIDAL ACTIVITY OF DIFFERENT COMPONENTS FROM HEMOLYMPH

PhD Student Venelin Tsvetkov, Assist. Prof. Daniel Todorov, PhD, Assist. Prof. Anton Hinkov, PhD, Assist. Prof. Kalina Shishkova, PhD, Prof. Stoyan Shishkov, PhD

Sofia University "St. Kliment Ohridski", **Bulgaria**

Following previous studies on the effect of hemocyanin subunits, the studies were continued. We studied different components of the hemolymph of several species of mollusks - *Carcinus aestuarii*, *Rapana cenosa*, *Helix lucorum*, *Eriphia verrucosa* and *Helix aspersa*. Experiments have been performed with these components on their effect on extracellular virions of human alphaherpes virus type 1 and human alpha herpes virus type 2 (labial and genital herpes).

The extracts with the most pronounced virocidal action are the mucus from *Helix aspersa* above 50 kDa - 99% HHV-1 inactivation was reported from the onset of contact and approximately 99% HHV-

2 inactivation approximately 240 minutes after the onset of contact. More than 99% inactivation of HHV-2 about the 240th minute of contact recorded in the hemolymph of *Eriphia verrusa* 3-100 kDa. The virocidal effect was determined by classical methodology by mixing equal volumes of virus suspension and test extract for different time periods (5, 10, 15, 30, 60, 120, 250 and 360 minutes) at 37 °C. Viral titer was determined on 48th hour by the method of Reed and Muench. The results are promising due to the presence of a virocidal effect and the possibility of using these natural substances both for the preparation of drugs and their inclusion in the composition of disinfectants.

Keywords: *Hemocyanin, HHV-1, HHV-2, Virucidal activity*

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This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018) under the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)”

Lecture 9. NMR SPECTROSCOPY FOR METABOLOMICS RESEARCH

Prof. Dr. Nikolay G. Vassilev¹, Prof. DSc. Svetlana D. Simova¹, Assist. Prof. Dr. Miroslav Dangalov¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Assist. Venceslav Atanasov¹, Assoc. Prof. Dr. Aleksandar Dolashki¹ and Prof. DSc. Pavlinka Dolashka¹

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Nuclear magnetic resonance (NMR) is one of the three principal analytical techniques used in metabolomics (the other two are gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled with single-stage mass spectrometry (LC-MS)). The relative ease of sample preparation, the ability to quantify metabolite levels, the high level of experimental reproducibility, and the inherently nondestructive nature of NMR spectroscopy have made it the preferred platform for long-term or large-scale clinical metabolomic studies. These advantages, however, are often outweighed by the fact that most other analytical techniques, including both LC-MS and GC-MS, are inherently more sensitive than NMR, with lower limits of detection typically being 10 to 100 times better. The advantages and disadvantages of NMR spectroscopy for metabolomic studies are discussed. A number of emerging NMR techniques and technologies are presented that are used to strengthen and overcome its inherent limitations in metabolomic applications.

An application of ¹H Nuclear Magnetic Resonance (NMR) spectroscopy for metabolic profiling was presented with the aim to investigate the functional role of the metabolites in lyophilized mucus from garden snail *Helix aspersa*. Twenty metabolites were unambiguously identified by ¹H, 1D TOCSY, 2D J-resolved, 2D COSY and 2D HSQC NMR spectra with water suppression. The metabolic profiles of two fractions with low molecular weight (Mw<1 kDa and Mw<3 kDa) are very similar. Metabolites with known antioxidant, antibacterial and antimicrobial activity have been detected by NMR metabolic analysis of mucus samples from *Helix aspersa*. Some of them were confirmed by mass spectrometric analysis.

Keywords: *mucus, garden snail Helix aspersa, ¹H NMR, Metabolites, mass spectrometry*

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POSTERS

Tuesday, 6th October 2020

Session: Ecological environment - water and air

P1. Assoc. Prof. Dr. Boyko Tsyntsarski, Ivanka Stoycheva, Georgi Georgiev, Temenuzhka Budinova, Kalina Miteva, Angelina Kosateva, Nartzislav Petrov, Slawomira Pusz, Urszula Szeluga, *POLYMER-BASED CARBON ADSORBENT FOR EFFECTIVE REMOVAL OF MERCURY FROM WATER*

P2. Assist. Dr. Ivanka Stoycheva, Boyko Tsyntsarski, Bilyana Petrova, Georgi Georgiev, Urszula Szeluga, Slawomira Pusz, *SYNTHESIS OF CARBON FOAM ON THE BASE OF MIXTURE OF COAL TAR PITCH AND FURFURAL WITHOUT USING PRESSURE AND STABILIZATION TREATMENT*

P3. Assist. MSc Georgi Georgiev, Ivanka Stoycheva, Temenuzhka Budinova, Boyko Tsyntsarski, Kalina Kotseva, Angelina Kosateva, Nartzislav Petrov, *CHARACTERIZATION AND APPLICATION OF NANOPOROUS CARBON FROM RDF WASTE FOR PHENOL REMOVAL*

P4. Dr. Angelina Kosateva, Ivanka Stoycheva, Bilyana Petrova, Boyko Tsyntsarski, *RAMAN SPECTRA OF CARBON ADSORBENTS, PRODUCED FROM REFUSE DERIVED ORGANIC FUEL*

P5. Dr. Gloria Issa, Martin Šťastný, Momtchil Dimitrov, Radostina Ivanova, Tanya Tsoncheva, *NANOSTRUCTURED MANGANESE OXIDE CATALYSTS IN CATALYTIC DECOMPOSITION OF METHANOL*

P6. Dr. Radostina Ivanova, Gloria Issa, Momtchil Dimitrov, Tanya Tsoncheva, *NANOSTRUCTURED MULTICOMPONENT OXIDE CATALYSTS FOR SUSTAINABLE ENVIRONMENTAL PROTECTION*

P7. Dr. Veronika Mihaylova, Valentina Lyubomirova, Romyana Djingova, *A COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION OF BULGARIAN BOTTLED MINERAL AND SPRING WATERS*

P8. Dr. Eng. Andrei Sârbu, Dr. Eng. Teodor Sandu, Dr. Eng. Tanta Verona Iordache, Dr. Eng. Anita Laura Chiriac, Prof. Dr. Boyko Tsyntsarski, Dr. Ivanka Stoycheva, Assist. Prof. Dr. Simona Caprarescu, Chim. Marinela Victoria Dumitru, Chim. Ana Lorena Ciurlica, Eng. Andreea Miron

P9. Albena Varsano¹, PhD student. *THE RETURN OF ART IN GREEN TECHNOLOGIES*

Wednesday, 07th October 2020

Session: Natural health products

P1. Assist. Prof. Maria Lazarova, Lyubka Tancheva, Pavlina Dolashka, Alexander Dolashki, Lyudmila Velkova, Albena Alexandrova, Elina Tsvetanova, Reni Kalfin, *AMELIORATING EFFECT OF SNAIL (HELIX ASPERSA) EXTRACT IN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE*

P2. Assist. Prof. Elina Tsvetanova, Albena Alexandrova, Almira Georgieva, Maria Lazarova, Pavlina Dolashka, Lyudmila Velkova, Alexander Dolashki, Venceslav Atanasov, Reni Kalfin, Lyubka Tancheva, *INFLUENCE OF SNAIL EXTRACT OF HELIX ASPERSA ON OXIDATIVE STRESS IN EXPERIMENTAL DEMENTIA*

P3. Assoc. Prof. Dr. Ivan Iliev, Elena Ivanova, Katerina Todorova, Ani Georgieva, Valeriya Dilcheva, Ivelin Vladov, Svetlozara Petkova, Reneta Toshkova and. Pavlina Dolashka, *HEMOCYANINS FROM HELIX LUCORUM, HELIX ASPERSA AND RAPANA VENOSA EXHIBIT ANTITUMOR ACTIVITY IN VITRO AND IN VIVO*

P4. Prof. DSc Spiro Konstantinov, MD PhD DSc, Assoc. Prof. Dr. Pavlina Dolashka, Maria Nikolova, Teodora Atanasova, *ANTINEOPLASTIC PROPERTIES OF HEMOCYANINS*

P5. Assistant Dr. Valeria Dilcheva, Ivelin Vladov, Ivan Iliev, Katerina Todorova, Ani Georgieva, Reneta Toshkova, Svetlozara Petkova, Pavlina Dolashka, *COMPARATIVE HEMATOLOGICAL STUDIES IN TRICHINELLA SPIRALIS AND TRICHINELLA PSEUDOSPIRALIS AFTER EXPOSURE TO HEMOCYANIN*

P6. Assis. Prof. Dr. Elena Krachmarova, Genoveva Nacheva, Kristina Malinova, Lyudmila Velkova, Aleksandar Dolashki, Ventseslav Atanasov, Pavlina Dolashka, *GASTROPOD EXTRACTS SHOW ANTIPROLIFERATIVE EFFECT IN VITRO*

P7. Alexander Tzintzarov, Zlatina Vlahova, Maria Petrova, Iva Ugrinova, *ANTITUMOUR ACTIVITY OF NATURAL PRODUCTS ISOLATED FROM SNAILS (HELIX ASPERSA, HELIX LUCORUM) AND RAPANA (RAPANA VENOSA)*

P8. Dr. Michaella Aleksova, Galina Radeva, *ANTIBACTERIAL ACTIVITY OF BIOACTIVE COMPOUNDS EXTRACTED FROM CRAB AND SNAIL SPECIES*

P10. Prof. Dr. Krassimira Yoncheva, Niko Benbassat, Maya M. Zaharieva, Lyudmila Dimitrova, Alexander Kroumov, Ivanka Spassova, Daniela Kovacheva, Hristo M. Najdenski, *ANTIMICROBIAL ACTIVITY OF OREGANO OIL ENCAPSULATED OF IN CHITOSAN-ALGINATE NANOPARTICLES*

P11. Eng. Marya Todorova, Aleksandar Dolashki, Penka Todorova, *DEVELOPMENT OF A NATURAL PRODUCT FOR PREVENTION AND SUPPRESSION OF SIDE EFFECTS IN CHEMOTHERAPEUTIC TREATMENT*

Thursday, 08th March 2020

Session: Ecological products and processes

P1. Prof. DSc Pavlina Dolashka, Lyudmila Velkova, Aleksandar Dolashki, Petya Hristova, *INVESTIGATION OF ANTIBACTERIAL POTENTIAL OF HELIX ASPERSA MUCUS BY TREATMENT OF PROPIONIBACTERIUM ACNES*

P2. Assoc. Prof. Dr. Lyudmila Velkova, Spiro Konstantinov, Aleksandar Dolashki, Ventseslav Atanasov, Pavlina Dolashka, *INVESTIGATION OF BIOACTIVE COMPOUNDS ISOLATED FROM MARINE SNAIL RAPANA VENOSA WITH POTENTIAL APPLICATION IN THE MEDICINE*

P3. Assistant MSc Ventseslav Atanasov, Maria Petrova, Jordana Todorova, Maria Schröder, Aleksandar Dolashki, Lyudmila Velkova, Iva Ugrinova, Pavlina Dolashka, *ANTITUMOR*

ACTIVITY OF BIOACTIVE COMPOUNDS FROM *RAPANA VENOSA* AND *HELIX ASPERSA* AGAINST TWO CANCER CELL LINES

P4. Assistand MSc Asya Daskalova, Lyudmila Velkova, Aleksandar Dolashki, Radoslav Abrashev, Nedelina Kostadinova, Ekaterina Krumova, Maria Angelova, Pavlina Dolashka, *ANTIOXIDANT AND ANTIFUNGAL PROPERTIES OF MUCUS FROM GARDEN SNAIL CORNU ASPERSUM*

P5. MSc Dimitar Kaynarov, Asya Daskalova, Ventseslav Atanasov, Lyudmila Velkova, Aleksandar Dolashki, Pavlina Dolashka, *ANALYSIS OF PROTEINS IN NATURAL EXTRACTS*

P6. Prof. Dr. Nikolay G. Vassilev, Svetlana D. Simova, Miroslav Dangelov, Lyudmila Velkova, Venceslav Atanasov, Aleksandar Dolashki and Pavlinka Dolashka, *A ¹H NMR AND MS BASED STUDY OF METABOLITES PROFILING OF GARDEN SNAIL HELIX ASPERSA MUCUS*

P7. Assistant Dr. Lyudmila Dimitrova, Maya M. Zaharieva, Krassimira Yoncheva, Niko Benbassat, Alexander Kroumov, Ivanka Spassova, Daniela Kovacheva, Hristo M. Najdenski, *OREGANO OIL ENCAPSULATED IN CHITOSAN-ALGINATE NANOPARTICLES INHIBITS THE FORMATION OF BACTERIAL BIOFILMS*

P8. Dr. Tsvetelina Doncheva, Nadezhda Kostova, Kalina Alipieva, Milena Popova, *GENTIANA CRUCIATA L. - SOURCE OF NEW BIOLOGICALLY ACTIVE TRITERPENE FRACTIONS*

P9. Dr. Maya Zaharieva, Lyudmila Dimitrova, Alexander Kroumov, Yana Ilieva, Krassimira Yoncheva, Martin Berger, Spiro Konstantinov, Hristo Najdenski, *MICELLAR CURCUMIN ENHANCES THE ANTIBACTERIAL ACTIVITY OF THE ALKYLPHOSPHOCHOLINE ERUFOSINE*

P10. Assoc. Prof. Lubomir Petrov, Albena Alexandrova, Elina Tsvetanova, Almira Georgieva, Pavlina Dolashka, Lyudmila Velkova, Alexander Dolashki, Venceslav Atanasov, *PROTECTIVE EFFECT OF MUCUS FROM HELIX ASPERSA ON ETHANOL INDUCED GASTRIC ULCERS IN MICE*

P11. Assoc. Prof. Dr. Olya Stoilova, *NATIONAL RESEARCH PROGRAMME "INNOVATIVE LOW-TOXIC BIOACTIVE SYSTEMS FOR PRECISION MEDICINE (BioActiveMed)"*

P12. Assoc. Prof. Dr. Aleksandar Dolashki, Lyudmila Velkova, Elmira Daskalova, Yana Topalova, Pavlina Dolashka, *CHARACTERISTICS OF NOVEL PEPTIDES OF CORNU ASPERSUM MUCUS WITH ANTIBACTERIAL ACTIVITY*

P13. Assoc. Prof. Dr. Krassimira Idakieva, Svetla Todinova, Aleksandar Dolashki, Lyudmila Velkova, Pavlina Dolashka, *CONFORMATIONAL STABILITY OF HEMOCYANIN ISOLATED FROM SNAILS HELIX LUCORUM*

P14. Assoc. Prof. Dr. Ekaterina Krumova, Pavlina Dolashka, Nedelina Kostadinova, Radoslav Abrashev, Jeny Miteva-Staleva, Aleksandar Dolashki, Lyudmila Velkova, Asya Daskalova, Vladislava Dishlijska, Boryana Spasova, Maria Angelova. *ANTIFUNGAL ACTIVITY OF THE HEMOLYMPH ISOLATED FROM MOLLUSKS AND ARTHROPODS*

ABSTRACTS POSTERS

SESSION 1: Ecological environment - water and air

P1. POLYMER-BASED CARBON ADSORBENT FOR EFFECTIVE REMOVAL OF MERCURY FROM WATER

Assoc. Prof. Dr. Boyko Tsyntsarski¹, Dr. Ivanka Stoycheva¹, Eng. Georgi, Georgiev¹, Prof. Dr. Temenuzhka Budinova¹, Eng. Kalina Miteva¹, Dr. Angelina Kosateva¹, Prof. Dr. Nartzislav Petrov¹, Assoc.Prof. Dr. Slawomira Pusz², Assoc.Prof. Dr. Urszula Szeluga²

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Adsorption of mercury ions on activated carbon, prepared from polymer waste, is studied. The structure and surface properties of the carbon sample are characterized by different methods - N₂ adsorption, IR spectroscopy, determination of surface oxygen groups. Adsorption studies of mercury from aqueous solution on synthesized activated carbon are investigated at different conditions - metal ion concentration, adsorbent dose, pH. It is established that the adsorption follows both Langmuir and Freundlich isotherms. The activated carbon obtained polymer waste demonstrates high adsorption capacity toward mercury ions (190 mg/g).

Keywords: *Water purification; activated carbon; porosity; polymer waste; mercury.*

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P2. SYNTHESIS OF CARBON FOAM ON THE BASE OF MIXTURE OF COAL TAR PITCH AND FURFURAL WITHOUT USING PRESSURE AND STABILIZATION TREATMENT

Dr. Ivanka Stoycheva¹, Assoc.Prof. Dr. Boyko Tsyntsarski¹, Dr. Bilyana Petrova¹, Eng. Georgi Georgiev¹, Assoc.Prof. Dr. Urszula Szeluga², Assoc.Prof. Dr. Slawomira Pusz²

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Carbon is an advanced porous material with multiple high-technology applications, due to its cell structure, light weight, high thermal stability, high thermal and electrical conductivity, as well as exceptional electromagnetic and acoustic properties. This work is dedicated to synthesis of carbon foam from a mixture of organic substances with suitable chemical properties and treatment conditions (the thermo-oxidative modification with H₂SO₄ and HNO₃ of a mixture of furfural and industrial coal tar pitch). Carbon foam is characterized by X-ray analysis, Scanning Electron Microscopy, TG and DSC analysis, Elemental analysis. The proposed new, less energy consuming synthesis method, allows to avoid the use of pressure and stabilization step, which enables the production of material with very good physico-chemical properties.

Keywords: Carbon foam; furfural; industrial coal tar pitch; XRD; TG

ACKNOWLEDGEMENTS

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P3. CHARACTERIZATION AND APPLICATION OF NANOPOROUS CARBON FROM RDF WASTE FOR PHENOL REMOVAL

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Phenol is one of the most common organic water pollutants, stable towards biodegradation. Phenol and substituted phenols are toxic organic pollutants, usually present in industrial waste waters, especially these from oil refineries, coal conversion plants, pharmaceuticals, etc.

Refuse-derived fuel (RDF) is a fuel produced from various types of waste such as municipal solid waste and industrial waste. RDF consists of combustible components, like polymers, paper and other waste materials. RDF can be also produced from used tyres or biomass waste. The combusted biomass fraction of RDF is used by stationary combustion operators to reduce their overall reported CO₂ emissions. Nanoporous carbons is prepared from RDF waste material by pyrolysis at 600 °C and subsequent hydro-pyrolysis at 800 °C. The synthesized carbon was characterized by N₂ physisorption at -196 °C, determination of oxygen-containing surface groups, IR spectroscopy, etc. Nanoporous carbon from RDF is characterized by high surface area of 600 m²/g and significant content of micro- and mesopores. The results suggest that obtained nanoporous carbon material is suitable for application as effective adsorbent of organic and inorganic pollutants.

Aqueous solutions with different initial known concentrations were used, as model water phenol solutions in the range from 100 mg/l to 300 mg/l. Adsorption isotherms were determined by using stoppered flasks, containing 0.1 g of carbon in 50cm³ of solution. They are agitated by a mechanical shaker for predetermined time intervals at room temperature to reach equilibrium conditions. The concentrations are determined spectrophotometrically, at maximum adsorption wavelength of 269 nm, using spectrophotometer Pfar0 300 UV spectrometer.

The Langmuir equation is applied to calculate the maximal adsorption capacity.

Nanoporous carbon with high surface area and well developed micro- and mesoporosity is obtained using solid waste from RDF as precursor. The obtained carbon show moderate adsorption capacity towards phenol in aqueous solution.

Keywords: adsorption; carbon; RDF; water purification; phenol.

ACKNOWLEDGEMENTS

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P4. RAMAN SPECTRA OF CARBON ADSORBENTS, PRODUCED FROM REFUSE DERIVED ORGANIC FUEL

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RDF fuel from municipal solid waste and the products obtained from its thermochemical treatment

were examined using a Raman microscope. The Raman spectral analysis was done using two-band (“G” at ~1578 and “D” at ~1340 cm^{-1}) and five-band (G, D1, D2, D3, and D4 at about 1580, 1350, 1500, 1620, and 1200 cm^{-1} respectively) combinations.

The results show the changes that occur in the chemical composition of the raw material in the stages of its treatments – thermo-chemical modification with a strong oxidizer and the subsequent carbonization of the resulting product. The formation of oxygen-containing compounds during the first stage and the formation of higher molecular compounds with aromatic character during the second stage have been established. Information was obtained on the nature of the surface of the materials.

Keywords: *Raman spectroscopy, refuse derived fuel (RDF), activated carbons*

ACKNOWLEDGEMENTS

This research was funded by project BG05M2OP001-1.002-0019:., Clean technologies for sustainable environment – water, waste, energy for circular economy“, financed by the Operational programme “Science and Education for Smart Growth” 2014-2020, co-financed by the European union through the European structural and investment funds.

P5. NANOSTRUCTURED MANGANESE OXIDE CATALYSTS IN CATALYTIC DECOMPOSITION OF METHANOL

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In the last decades, the design and synthesis of nanostructured metal oxides has gained much attention due to their attractive physical, chemical, and catalytic properties. Manganese oxides are known to be active catalysts in several oxidation or reduction reactions. The catalytic activities of manganese oxides catalysts are dependent upon the valence states of manganese as well as the participation of active oxygen species, oxygen vacancy, and lattice oxygen in the catalytic reactions. In particular, Mn_xO_y have been reported as the most efficient and environmentally friendly materials for catalytic decomposition of methanol at low temperatures. An increase in catalytic activity was observed at occurrence when Mn^{3+} and Mn^{4+} were coupled in the manganese oxide structure. In this work, a series of five manganese oxides-based catalysts were prepared by homogeneous precipitation accompanied by redox reactions of manganese salts in an aqueous solution. The prepared catalysts were characterized by complex of various physicochemical techniques, such as nitrogen physisorption (BET), XRD, HRTEM, H_2 -TPR, and Raman spectroscopy. The activity of nanostructured manganese oxides (MnO_x) were investigated in the catalytic decomposition of methanol. The catalytic activity and selectivity of the synthesized manganese oxide materials could be successfully controlled by the preparation procedure and the specific characteristics of the acidic and redox-active sites in them. Depending on the mechanism of the catalytic reaction, the catalytic activity is in a complex relation to the oxidative state, particle dispersion, and morphology, which are controlled by the preparation procedure and the precursor used.

Keywords: *Nanostructured manganese oxide catalyst, physicochemical characterization, catalytic decomposition of methanol*

ACKNOWLEDGEMENTS

The authors thank to the project KII-06-H29/2 for the financial support and bilateral project BAS-AS CR for the help with the physicochemical characterization of the samples.

P6. NANOSTRUCTURED MULTICOMPONENT OXIDE CATALYSTS FOR SUSTAINABLE ENVIRONMENTAL PROTECTION

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Transition metal oxides are involved as the main active component in a number of industrial catalysts, but their lower catalytic activity compared to noble metals is their main drawback. A well-known approach to increase their catalytic activity is the development of nanodispersed mixed oxide materials with mesoporous structure. The aim of the current investigation is to study the possibilities for obtaining new nanostructured mesoporous multicomponent catalysts based on cerium, manganese and titanium oxides, promoted with oxides of copper, iron and cobalt in several aspects: 1) in order to clarify the effect of the formation of solid solutions or mixed oxide surface layers with a high degree of defectiveness on the specific properties of mono- and bi-component cerium, manganese and titanium oxides; 2) in order to follow the possibilities for tuning the state of the supported copper, iron and cobalt oxide particles; 3) in order to follow the possibilities for their application as catalysts in reactions of ecological importance (total oxidation of ethyl acetate and methanol decomposition). The results from the catalytic tests show that the modification with copper and iron oxide phase leads to an increase in the catalytic activity of the supports. The results of the physicochemical characterization of the materials show the formation of finely dispersed and easily reducible active phases. Modification with cobalt oxide phase does not improve the catalytic activity of the supports, which is probably due to the blocking of the active phase and the formation of a hardly reducible under these conditions Co₃O₄ spinel phase.

Keywords: *transition metal oxides, ethyl acetate oxidation, physicochemical characterization*

ACKNOWLEDGEMENTS

The authors thank to the project KP-06-PM39/1/2019 for the financial support.

P7. A COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION OF BULGARIAN BOTTLED MINERAL AND SPRING WATERS

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Water makes up 60-75% of human body weight and is the most consumed natural product. It is the only zero-calorie, zero-sugar and additive-free beverage that will ensure a healthy form of hydration. Most people in the EU have good access to high quality drinking water. According to the European Federation of Bottled Waters, Bulgaria is one of the first countries in the consumption of bottled waters per capita for drinking purposes. It is also worth mentioning that within the EU, the consumption of bottled mineral water surpasses many times that of spring waters.

In the present study, using inductively coupled plasma mass spectrometry (ICP-MS) the concentrations of 69 chemical elements was determined for the first time in mineral and spring waters sold on the Bulgarian market. The obtained data complement the parameters determined according to the Bulgarian and European legislation (pH, conductivity, limited number of macroelements, etc.) and are a good basis for the correct choice of suitable drinking water, both for daily needs and after medical recommendation.

Keywords: *mineral and spring water, chemical composition, ICP-MS.*

ACKNOWLEDGEMENTS

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P8. ADVANCED MEANS FOR HEAVY METALS REMOVAL

Dr. Eng. Andrei Sârbu¹, Dr. Eng. Teodor Sandu¹, Dr. Eng. Tanta Verona Iordache¹, Dr. Eng. Anita Laura Chiriac¹, Prof. Dr. Boyko Tsyntsarski², Dr. Ivanka Stoycheva², Assist. Prof. Dr. Simona Caprarescu³, Chim. Marinela Victoria Dumitru¹, Chim. Ana Lorena Ciurlica¹, Eng. Andreea Miron¹

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One of the major problems, we are facing nowadays is water pollution. In this context, it becomes worth mentioning wastewaters released by various industry branches [1, 2]. These wastewaters bear either dyes or heavy metals, which pose significant environment challenge affecting the people health. Heavy metals are non biodegradable and thus polluting, becoming necessary identifying viable means of their removal from wastewaters. This work provides an innovative and efficient approach that uses a combination of two different principles: polymer membranes and carbon powders. For a thorough study, polymer membranes were prepared by phase inversion, using three copolymers of acrylonitrile, with different co-monomers. Moreover, two different carbon powders, of natural and synthetic origin, were used in order to obtain composite membranes. It was found that final properties (water permeation and thermal behavior) of membranes may be pretty well adjusted by changing either the co-polymer or the carbon powder. Moreover, the obtained membranes were proved to achieve an advanced heavy metals removal, as shown by tests carried out with nickel-laden wastewaters.

1. Lewis SR, Datta S, Gui M, Coker EL, Huggins FE, Daunert S, Bachas L and Bhattacharyya D, *PNAS* **108**: 8577- 8582 (2011).
2. Huang Y, Miao YE and Liu T, *J. Appl. Polym. Sci.* DOI: 10.1002/APP. 40864 (2014)

Keywords: *polymer membranes, carbon-polymer composites, water purification, heavy metals.*

ACKNOWLEDGEMENTS

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P9. THE RETURN OF ART IN GREEN TECHNOLOGIES

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The new understanding of sustainable business, products and technologies includes the ancient sense of *techne*- interpreted by Heidegger as the lost art and impulse of creation. Environmentally sustainable products or technologies are expected to effectively integrate all four sustainable pillars: human, social, economic and environmental (Dunphy, 2000). The ethics of this approach could be seen as a result of the fact that scientists face that the environmental problems, they need to address

require human, social and cultural transformation at least as much as scientific and technological (Gus Speth). To transform the ethical principles into practical solutions the gap between exact and experimental studies and humanitarian studies should be filled at local level as a reply to world processes and tendencies.

There is a variety of methods that could enforce the achievement of this goal:

- Ecocriticism as an interdisciplinary point of view that studies literature and the environment;
- Ecolinguistic as a method to observe how language transfer ecological ideas and how language affects environmental literacy and intelligence (Alwin Fill);
- Public participation methods that include different social groups into the process of science and technology transfer and implementation.

The result of this ‘translation’ process will be to inform, raise consciousness and engage the wide audience to particular environmental problems as well as to help them understand particular decision or technology and its expected effects on human, society, the environment and on economics.

The Center of Competence “Clean&Circle” and similar scientific and educational hubs could be excellent providers and mediators in this process, linking business, science, education and society.

Keywords: *sustainable development, ecocriticism, public participation, art*

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SESSION 2: Natural health products

P1. AMELIORATING EFFECT OF SNAIL (*HELIX ASPERSA*) EXTRACT IN EXPERIMENTAL MODEL OF PARKINSON’S DISEASE

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Molluscs have been a significant focus in the search for biologically active secondary metabolites with potential to be a source of new drugs. For centuries molluscs as snail for example have been used as a food and as treatment for a variety of medicinal conditions. One of the most common therapeutic applications of molluscs in traditional ethnomedicine appears to be for treatment of inflammatory conditions. Inflammation itself is associated with the pathogenesis of many acute and chronic diseases, including neurodegenerative disorders (Nathan and Ding, *Cell*, 140 (6), 871–882, 2010). Hence, the aim of the present study was to check potential protective and therapeutic effect of snail extract on Parkinson’s disease (PD) experimental rat model.

Snail extract was collected and purified from *Helix aspersa* snails, grown in Bulgarian farms using patented technology without suffering any snail (Dolashka et al., *BG Useful model* 2097, 2015). Experimental rat PD model was induced via 6-OHDA unilateral striatal injection (10 µg/2µl) as previously described (Lazarova et al., *J. Mol Neuroscience* 66(4), 552-560, 2018). Male Wistar rats were divided into following groups: sham-operated, striatal 6-OHDA-lesioned control group and 6-OHDA-lesioned rats treated for 13 days with fresh water snail extract orally (0.5 ml/100 g body weight, 6 days before and 7 days after striatal lesion). On the 1st, 2nd and 3rd week post lesion rats

were subjected to behavioral tests: apomorphine-induced rotation test, rotarod test and step-through test. After that brains were removed and studied for changes for oxidative status indexes. Lipid peroxidation, glutathione levels and antioxidant enzymes activities were estimated in brain homogenates. In PD group apomorphine-induced rotations, number of falls and memory impairment increased significantly as compared to sham-operated group, which verifies PD experimental rat model. The treatment with snail extract reduced significantly apomorphine-induced rotations (by 46%), number of falls (by 90 %) and compensated memory deficit by 23 % as compared to 6-OHDA control group. These changes were accompanied by significant decrease in brain lipid peroxidation and recovery of brain superoxide dismutase activities, increased by 6-OHDA-treatment.

In conclusion the snail extract exerted convincing beneficial effect on experimental rat model of Parkinson's disease confirmed both behaviorally and biochemically.

Key words: *Snail extract, Parkinson's disease, Memory*

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P2. INFLUENCE OF SNAIL EXTRACT OF *HELIX ASPERSA* ON OXIDATIVE STRESS IN EXPERIMENTAL DEMENTIA

Assist. Prof. Elina Tsvetanova¹, Prof. Albena Alexandrova^{1,2}, Dr. Almira Georgieva¹, Assist. Prof. Maria Lazarova¹, Prof. DSc. Pavlina Dolashka³, Assoc. Prof. Dr. Lyudmila Velkova³, Assoc. Prof. Dr. Alexander Dolashki³, Assist. Venceslav Atanasov³, Prof. Reni Kalfin¹, Prof. Lyubka Tancheva¹

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Terrestrial slugs and snails produce mucus which is a rich source of bioactive natural compounds that are used for the treatment of wounds, burns, psoriasis, acne, wrinkles, skin damages. Moreover, it was shown that the antioxidant potential of this mucus may due to allantoin (Gushkov et al., Doklady Biochemistry and Biophysics, 383, 105, 2002), and might possess a therapeutic potential for the cognitive dysfunctions observed in Alzheimer's disease (Ahn et al., Food Chem Toxicol 64, 210, 2014). Hence, the aim of this study was to evaluate the effect of snail (*Helix aspersa*) mucus extract on oxidative stress in rat brain cortex in an experimental model of scopolamine-induced dementia of Alzheimer's type. Scopolamine was applied i.p. (2 mg/kg) in male Wistar rats for 11 days along with peroral administration of snail mucus extract (0.5 mL/100 g). On the 12th day cortex was isolated and oxidative stress parameters: lipid peroxidation (LP), total glutathione (GSH), activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed spectrophotometrically. Scopolamine treatment provoked increase in LPO, CAT and GPx activities and decreased tGSH and SOD activity.

According to our results we could assume that the activation of antioxidant enzymes in response to scopolamine-induced oxidative stress is a cellular protective mechanism. Although the obtained results could not provide clear understanding of the mechanism of action of the snail extract, whether it acts directly or indirectly, we could hypothesized that the snail extract is able to affect positively the impaired brain cognitive functions, which effect we clearly have demonstrated in another study. Due to received evidence our suggestion is that snail extract acts as antioxidant and stimulates important stress response pathways in cell affecting endogenous cellular antioxidant levels and diminishing the neurodegenerative processes in dementia of Alzheimer's type.

Key words: *Snail extract, Oxidative stress, Alzheimer's disease, Dementia*

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This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018) under the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)” approved by DCM # 658 / 14.09.2018.

P3. HEMOCYANINS FROM *HELIX LUCORUM*, *HELIX ASPERSA* AND *RAPANA VENOSA* EXHIBIT ANTITUMOR ACTIVITY *IN VITRO* AND *IN VIVO*

Assoc. Prof. Dr. Ivan Iliev¹, Bachelor Elena Ivanova¹, Chief Assist. Prof. Dr. Katerina Todorova¹, Chief Assist. Prof. Dr. Ani Georgieva¹, Chief Assist. Prof. Dr. Valeriya Dilcheva¹, Chief Assist. Prof. Dr. Ivelin Vladov¹, Prof. Dr. Svetlozara Petkova¹, Prof. Dr. Reneta Toshkova¹ and Prof. DSc. Pavlina Dolashka²

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Hemocyanins are respiratory, oxygen-carrying metalloproteins that are freely dissolved in the hemolymph of mollusks, cephalopods and arthropods. The Interest in hemocyanins has grown significantly since it was found that they can be successfully used in immunotherapy of tumors as non-specific or active stimulators of the immune system.

The aim of the present study was to determine the potential of hemocyanins isolated from marine snail *Rapana venosa*, garden snails *Helix lucorum* and *Helix aspersa*, as well the mucus of *H. aspersa* for antitumor therapy. The antitumor effect of the above hemocyanins and their derivatives were studied under *in vitro* and *in vivo* conditions. *In vitro* experiments were performed on a panel of cell lines - a model of breast cancer. The effects of the hemocyanins on the cell viability were analyzed by MTT assay. Ehrlich Ascites Carcinoma is a mouse breast cancer and was used as a model of tumor disease *in vivo*.

The high antitumor activity of α -HaH determined under *in vitro* conditions was also observed in *in vivo* experiments. Evaluation of *in vitro* cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay performed after 24 h of incubation shows that the tested hemocyanins are non-toxic. In the *in vivo* experiments, the results of histochemical analysis (E&H), examining organ and systemic toxicity observed that there were no acute, subacute or chronic toxic effects in the treated animals.

The presented data indicate that hemocyanins are a promising class of anticancer therapeutics due to their immunogenic properties and lack of toxicity or side effects.

Keywords: *hemocyanins, cytotoxicity, antitumor activity*

ACKNOWLEDGEMENT

This study was supported by contract № Д01-323/18.12.2019 г., Program "Innovative Low-Toxic and Biologically Active Means for Precision Medicine" – BioActiveMed. Ministry of Education and Science of the Republic of Bulgaria.

P4. ANTINEOPLASTIC PROPERTIES OF HEMOCYANINS

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Nowadays, malignant diseases are one of the major public health problems. There is no single “cure for cancer” and traditional types of treatment (surgery, chemotherapy, radiation therapy, etc) are still not effective enough. Last decades a lot of natural products demonstrated strong cytotoxic effect against tumor cell lines without causing damage to not malignant cells.

Hemocyanins are oxygen-transporting, copper containing glycoproteins, freely dissolved in the hemolymph of many arthropods and mollusks. Molluscan hemocyanins are among the largest known proteins, with molecular masses varying approximately from 3.3 to 13.5 MDa. They form decamers or multi-decamers of individual polypeptide subunits of 330–450 kDa, with each subunit consisting of seven or eight functional units (~50 kDa). These bioactive substances are not only transport proteins for oxygen, but they also exert antineoplastic, antiviral, immune-modulating activities and phenoloxidase –like functionality. *Keyhole Limpet* hemocyanin (KLH) is one of the most popular and studied proteins which boost and inspire future tests with other effective hemocyanins such as *Concholepas concholepas* hemocyanin and *Fissurella latimarginata* hemocyanin. In preliminary studies in our research group *Rapana venosa* hemocyanin and *Helix aspersa* hemocyanin showed antineoplastic and immune-modulating properties similar to KLH. Therefore we performed experiments with sterile extracts isolated from snails and *Rapana venosa* and demonstrated antineoplastic effects against different malignant cell lines as measured by the colorimetric MTT assay. The panel of cell lines included following cell lines: T-24 urinary bladder cells, Hut-78 (Sezary Syndrome), MJ –cells (cutaneous T cell lymphoma; mycosis fungoides) and CAL-29 (urinary bladder transitional cell carcinoma). Antineoplastic activity significantly occurs at 1000 µg/ml concentrations. Notable cytotoxic effect appeared at treated T-24 urinary bladder cells with α-subunit of *Helix aspersa* (α-HaH) at concentration 1000 µg/ml – almost 80% inhibition of cell viability. Treated Hut-78 (Sezary Syndrome) cells with α-HaH, β-subunit of *Helix aspersa* (β-HaH) and subunit-1 of *Rapana venosa* hemocyanin (Rv H I) at concentration 1000 µg/ml reached 60% reduction of cells viability. Hemolymph of *Rapana venosa* 30-100 kDa at concentration 1000 µg/ml caused almost 90% inhibition of MJ-cell survival. Also, extract of hemolymph *Rapana venosa* 50-100 kDa demonstrated 80% inhibition of cell viability to CAL-29 cell line.

Taken together our experimental data indicates that hemocyanins have concentration dependent antineoplastic activity *in vitro* against variety of tumor cells. Because of the very complex glycoprotein structure (mannose rich glycosylation) of hemocyanins immune-stimulating properties can be expected as well. There is another potential of hemocyanins to modulate cell adhesion, which may contribute to the inhibition of the tumor growth. Hemocyanins lack of any common toxicity thus being appropriate for use in humans with locally controllable malignant diseases, such as non-invasive urothelial carcinoma and cutaneous T-cell lymphoma.

Keywords: Hemocyanins, antineoplastic activity, *Helix aspersa*, *Rapana venosa*

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217) under the National Research Programme “ Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)”.

P5. COMPARATIVE HEMATOLOGICAL STUDIES IN TRICHINELLA SPIRALIS AND TRICHINELLA PSEUDOSPIRALIS AFTER EXPOSURE TO HEMOCYANIN.

Chief. ass. Dr. Valeria Dilcheva¹, Chief. ass. Dr. Ivelin Vladov¹, Assoc. Prof. Dr. Ivan Iliev¹, Chief. ass. Dr. Katerina Todorva¹, Chief. ass. Dr. Ani Georgieva¹, Prof. Dr. Reneta Toshkova¹, Prof. Dr. Svetlozara Petkova¹, Prof. DSc. Pavlina Dolashka²

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Hemocyanins are oxygen-transporting glycoproteins in the hemolymph of arthropods and mollusks that due to their proven immunostimulatory characteristics and the lack of toxic side effects attract scientific interest in pharmacy and clinical medicine.

The study aimed to observe hematological changes occurring during experimentally induced infection with *Trichinella spiralis* and *T. pseudospiralis* in mice after exposure to hemocyanins isolated from marine snail *Rapana venosa* (RvH), garden snails *Helix lucorum* (HIH) and *Helix*

aspersa (HaH), and the mucus of *H. aspersa* snails. We performed hematological blood profile, tracking 15 blood indicators. In statistical analysis made by Two-way ANOVA, of hematological parameters in two types of trichinellosis compared to control animals.

Keywords: *hemocyanins, Trichinella spiralis, Trichinella pseudospiralis, immunostimulatory*

ACKNOWLEDGEMENTS

This work was supported by the Bulgarian Ministry of Education and Science (Grant D01-217) under the National Research Programme “ Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed) ” .

P6. GASTROPOD EXTRACTS SHOW ANTIPROLIFERATIVE EFFECT *IN VITRO*

Assist. Prof. Dr. Elena Krachmarova¹, Assoc. Prof. Dr. Genoveva Nacheva¹, Kristina Malinova¹, Assoc. Prof. Dr. Lyudmila Velkova², Assoc. Prof. Dr. Aleksandar Dolashki², Assist. Ventseslav Atanassov², Prof. DSc. Pavlina Dolashka²

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At present mucus and haemolymph from garden snails are well known for their antimicrobial activity and skin regeneration properties. Tissue extracts from different *Helix* species have been shown to exhibit anticancer activity against breast cancer cells, erythromyeloid leukemia-derived cells and colorectal adenocarcinoma cells. In this study the antiproliferative effect of mucus and hemolymph from *Helix aspersa* and *Helix lucorum* was studied on WISH cell line. The transformed aneuploid WISH cell line was derived from human amniotic epithelium, that combines features of normal amniotic cells with cervical cancer cells. Low molecular weight fraction from *Helix aspersa* mucus (3-10 kDa) showed about 30 % inhibition effect on the growth of WISH cells. The remarkable 80 – 85 % cytotoxic effect was observed when the cells were treated with peptide fraction from hemolymph from *Helix lucorum* with molecular weight from 1 to 10 kDa.

To explain the observed activity, the peptide fractions were purified by reversed phase high performance liquid chromatography (RP-HPLC). Using tandem mass spectrometry MALDI-TOF-MS/MS, the primary structures of 10 novel peptides in the active fractions were identified. The most of them contain high levels of glycine and leucine residues, but others contain proline, tryptophan and valine residues, typical for peptides with antimicrobial activity.

For the first time *in vitro* antiproliferative effect of peptides from mucus and hemolymph from *H. aspersa* and *H. lucorum* against malignant WISH cell line was found. These results may be considered as basic information for further investigations of anticancer activity of novel bioactive compounds from garden snails.

Keywords: *gastropod extracts, WISH cells, antiproliferative effect*

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P7. ANTITUMOUR ACTIVITY OF NATURAL PRODUCTS ISOLATED FROM SNAILS (*HELIX ASPERSA*, *HELIX LUCORUM*) AND RAPANA (*RAPANA VENOSA*)

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With the progress of anticancer therapy in recent years, finding of selective and more efficient new drugs is one of the greatest challenges for biomedicine and pharmacology. The identification of new target molecules and processes imposes a shift in the focus of the therapeutic approaches – from

broad spectrum cytotoxic agents to more specific analogues and/or methods tailored to the type of cancer and pathological context of the patient. This approach helps the prevention of numerous negative effects of the traditional therapy including side effects on healthy tissues and multidrug resistance. The numerous structural and functional studies on natural products isolated from snails suggest the latter as a good candidate for the development of novel low-toxicity remedies and therapeutic strategies.

The aim of the study is to evaluate the cytotoxic activity of natural products with low toxicity isolated from snails (*Helix aspersa*, *Helix lucorum*) and Rapana (*Rapana venosa*) - native hemocyanins, hemocyanin subunits and hydrolysates. Molluscan hemocyanins (Hcs) are oligomeric copper-containing glycoproteins that function as oxygen carriers in the hemolymph of mollusk and arthropod. Using various human cancer cell lines, with a different profile and degree of expression of oncogenes and/or drug resistance genes, we conducted an *in vitro* cell-based assay in which the cells were treated with Hcs. Half-maximal inhibitory concentration (IC₅₀) of the Hcs was obtained from an experimentally derived dose-response curve.

Three of the studied products – total hemocyanin and two different hemocyanin subunits exhibited anti-tumor activity.

Keywords: *natural products; mollusc; hemocyanins; anti-tumour activity; half-maximal inhibitory concentration (IC50)*

ACKNOWLEDGMENTS

This work was fully supported by the Bulgarian Ministry of Education and Science (Grant D01-217/30.11.2018) under the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)” approved by DCM # 658 / 14.09.2018.

P9. ANTIBACTERIAL ACTIVITY OF BIOACTIVE COMPOUNDS EXTRACTED FROM CRAB AND SNAIL SPECIES

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Increasing antibiotic resistance to clinically significant bacteria has become a global problem in the recent years. Hemolymph and mucus of *Helix lucorum* and *Helix aspersa* garden snails and *Rapana venosa* marine snail, as well hemocyanin from *Carcinus aestuarii* are a complex mixture of bioactive components with promising application in treatment of pathogenic bacteria.

In this study, six different snails and crab bioactive compounds, extracted from the garden snails *Helix aspersa* and *Helix lucorum*, the marine snail *Rapana venosa* and from *Carcinus aestuarii* were evaluated for their antibacterial activity. The latest were tested against the following Gram negative bacterial strains: *E.coli* HB101 (DSM1607), *E.coli* (DSM1116) and *Brevundimonas diminuta* (DSM 1635) by Minimum Inhibitory Concentration (MIC) method. The strains are in the list for antibiotic susceptibility testing according to DSMZ German collection of microorganisms and cell cultures GmbH.

Our results showed that the dissociated hemocyanin from *C. aestuarii* (9.29 mg/ml) inhibits the growth of the bacteria even at dilutions in range between 16 to 32 times of the initial concentration. The second compound mucus from *H. aspersa* (Mw 20 kDa - 2.73 mg/ml) showed a selective inhibitory effect on *E. coli* DSM1607, while the hemolymph from *H. lucorum* (Mw 10 kDa - 1.726 mg/ml) inhibited *Br. diminuta*. In conclusion, the hemocyanin exhibited very strong antibacterial activity and was capable of completely inhibiting the development of the tested bacterial strains. For further research a wide range of Gram negative bacterial strains is requested to confirm the antibacterial activity of the studied bioactive compounds.

Keywords: *antibacterial activity, Gram negative bacteria, minimum inhibitory concentration*

ACKNOWLEDGEMENTS

The authors acknowledges the support of the National Scientific Program BioActivMed (D01-217) by the Bulgarian Ministry of Education.

P10. ANTIMICROBIAL ACTIVITY OF OREGANO OIL ENCAPSULATED OF IN CHITOSAN-ALGINATE NANOPARTICLES

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Aim of the study is to load oregano oil in delivery systems based on hydrophilic vehicles in order to expand its therapeutic impact in the treatment of bacterial and fungal infections. Nanosystems with oregano oil can be used successfully also in the food industry for preservation of meat products. The encapsulation of the oregano oil into chitosan-alginate nanoparticles was performed by emulsification and consequent electrostatic gelation of both biopolymers. Physicochemical characterization of the oil loaded chitosan-alginate nanoparticles included determination of their size, FTIR spectroscopy, thermogravimetric and XRD analyses. The microbicidal effect and the bacterial respiratory activity were determined by ISO 20776-1:2006(E) and MTT tests, respectively. Seven pathogenic bacterial and one fungal strains were evaluated. The data were analysed with the GraphPad Prism software. The physicochemical characterization showed that the oil loaded nanoparticles had small size and negative charge. The data from FTIR spectroscopy and XRD analyses revealed successful encapsulation of the oil into the polymer nanoparticles. The results from thermogravimetry suggested an improved thermal stability of the encapsulated oil. The oregano oil exhibited strong antimicrobial activity. The MICs of the encapsulated oil were up to ten fold lower than that of the pure oil. The polymer nanoparticles inhibited the respiratory activity of the bacterial strains to a lower extend than the pure oil but the bacterial growth inhibition occurred at significantly lower concentrations than those of the pure oil. The encapsulated oregano oil possesses strong antimicrobial activity and is promising for development as a food additive.

Keywords: *oregano oil, biopolymers, antimicrobial activity*

ACKNOWLEDGEMENTS

This study was supported by the National Scientific Program "Innovative low-toxic biologically active agents for precision medicine (BioActiveMed)" (Decision of the Council of Ministers of Bulgaria No. 658/14.09.2018).

P11 . DEVELOPMENT OF A NATURAL PRODUCT FOR PREVENTION AND SUPPRESSION OF SIDE EFFECTS IN CHEMOTHERAPEUTIC TREATMENT

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The activity of "BusinessLab" Ltd. is aimed at solving important problems in the field of public health through the application of research and development.

Conventional treatment of various malignancies includes mainly surgical, radiation and chemotherapeutic therapy. The treatment applied often causes various harmful side effects for the patients and it is even more worrying that in many cases the tumor cells develop resistance to therapeutic agents. For this reason, the search for new natural products is the goal of many laboratories around the world.

To develop its entrepreneurial idea of creating an innovative product to prevent the side effects of chemotherapy, BusinessLab Ltd. applied under the Operational Program "Innovation and Competitiveness" 2014-2010, scheme BG16RFOP002-2.024 "Promotion of Entrepreneurship", co-financed by the European Union through the European Regional Development Fund and won the project "Development of a natural product for prevention and suppression of side effects in

chemotherapeutic treatment ”BG16RFOP002-2.024-0058-C01 / 09.08.2019. The goals of the project will be achieved by a team with extensive scientific experience, which participates in the creation and implementation of innovative products on the market, for which it receives a number of awards, such as Pythagoras 2016 and Pythagoras 2018.

The main goal of the project is to create an innovative natural product to prevent the side effects of chemotherapy (ProtHimoTherapy). This will be achieved through the development of active components of snails and calendula by a highly qualified team, through the application of innovative approaches and the use of chromatographic, mass spectrometric, microscopic and other scientific methods.

Another important task of the project proposal is to develop environmentally friendly technologies for the preparation of extracts and their combined application in the preparation of ProtHimoTherapies, a wide range of actions and in accordance with goal 1 of the priority axis "Biotechnology, with direct application to healthy living». The result of the project is to develop an innovative technology to offer a product ProtHimoTherapy, which has a lasting synergistic effect - antimicrobial, restorative and protects against the side effects of chemotherapy, which are a pandemic of our time.

Keywords: *innovative natural product, ProtHimoTherapy,*

ACKNOWLEDGEMENTS:

This work was supported by the project BG16RFOP002-2.024-0058-C01/09.08.2019 "Development of a natural product for prevention and suppression of side effects in chemotherapeutic treatment", funded by the Operational Program "Innovation and Competitiveness" 2014-2020, scheme BG16RFOP002-2.024 "Entrepreneurship Promotion ", Co-financed by the European Union through the European Regional Development Fund.

SESSION 3: Ecological products and processes

P1. INVESTIGATION OF ANTIBACTERIAL POTENTIAL OF *HELIX ASPERSA* MUCUS BY TREATMENT OF *PROPIONIBACTERIUM ACNES*

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Acne vulgaris is one of the most common inflammatory skin disease which is observed in great number of young people. The bacterium *Propionibacterium acnes* appears to play a critical role in the induction of inflammation. Recent microbiological and dermatological studies have reproducibly pointed out the strong associations between *P. acnes* and acne vulgaris, *S. aureus* and atopic dermatitis, and Malasseziasespecies with dandruff. Resistance development in *P. acnes* is another serious problem using conventional antibiotics such as erythromycin. In these contexts, we investigated several mucus extracts from garden snail *Helix spersa* for antibacterial activity.

The isolated mucus extract was separated into several fractions by ultrafiltration on Millipore membrane filters from 10 kDa, 20 kDa, 30 kDa and 100 kDa. The obtained fractions were tested for antimicrobial activity by the well diffusion method against *Propionibacterium acnes* 266 (AI).

The fraction having below 20 kDa displayed the highest antibacterial activity against *P. acnes* 266 (AI), followed from fraction above 100 kDa. Using tandem mass spectrometry we identified the primary structures of many novel antimicrobial peptides in active fraction below 20 kDa. Most of them contain high level of glycine, proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity. We have applied a combination of two-dimensional electrophoresis (2-DE) and mass spectrometry (MALDI-MS) to identify changes in expression of proteins secreted by *P. acnes* 266 (AI), before and after treatment with active fraction below 20kDa. The proteomic analysis showed that treatment with active fraction leads to a significant decrease in the expression of a number of proteins associated with cell adhesion and virulence of *P. acnes*, such as secretory proteins PPA1715, PPA1939, PPA2164, lipolytic enzymes in the secretory fraction, including triacylglycerol lipase, (GehA; PPA2105), but also succinyl-CoA synthetase subunit alpha, PPA1754; putative adhesion or S-layer protein, PPA2127 and CAMP factor 2 (PPA0687).

Keywords: *bacterium Propionibacterium acnes, mucus extracts, Helix spersa, antibacterial activity*

ACKNOWLEDGEMENTS

This research was carried out with the support of a project under contract No. DN 01/14 of 19.12.16, funded by the Scientific Research Fund of the Ministry of Education and Science, Bulgaria.

P2. INVESTIGATION OF BIOACTIVE COMPOUNDS ISOLATED FROM MARINE SNAIL *RAPANA VENOSA* WITH POTENTIAL APPLICATION IN THE MEDICINE

Assoc. Prof. Dr. Lyudmila Velkova¹, Prof. Dr. Spiro Konstantinov², Assoc. Prof. Dr. Aleksandar Dolashki¹, Assist. Ventseslav Aatanasov¹, Prof. DSc. Pavlina Dolashka¹

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The priority for the next years will be centered on investigation of alternative biomolecules with a new mechanism of action for therapy and enhancing human longevity. The aim of this study was to investigate the anti-tumor effect of different fractions from hemolymph of *Rapana venosa* which is a complex mixture from various bioactive substances.

The hemolymph from *R. venosa*, was separated into several fractions, by ultrafiltration using membranes with different pore sizes. The native molecule of *R. venosa* hemocyanin (RvH) and its two structural subunits RvH1 and RvH2 were isolated and purified from fraction >100 kDa. The isolated components: RvH, RvH1 and RvH2, fraction below 10 kDa and fraction between 10-50 kDa were investigated *in vitro* for antitumor activity of human cell lines: bladder cancer (T-24) and cutaneous T-cell lymphomas (MJ, Mycosis fungoides and HuT-78, Sézary syndrome).

The results revealed a significant cytotoxic effect of structural subunit RvH2 against bladder cancer (tumor cells line T-24). The most effective inhibition against cutaneous T-cell lymphomas (MJ, Mycosis fungoides) was observed after treatment with fraction, containing bioactive components with Mw between 10-50 kDa from hemolymph of *R. venosa*.

The absence of toxic effects on *R. venosa* extracts makes them promising candidates for topical application in the bladder before and after transurethral resection. Our results may be considered as basic information for further investigations on bioactive compounds from hemolymph of *R. venosa* for creating new natural products with potential biomedical applications.

Keywords: *marine snail Rapana venosa, hemolymph, R. venosa hemocyanin, anti-tumor activity*

ACKNOWLEDGEMENTS

This work was supported by the National Research Programme “Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)” approved by DCM № 658/14.09.2018 funded by the Bulgarian Ministry of Education and Science

P3. ANTITUMOR ACTIVITY OF BIOACTIVE COMPOUNDS FROM *RAPANA VENOSA* AND *HELIX ASPERSA* AGAINST TWO CANCER CELL LINES

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The present study is shown *in vitro* investigation and an evaluate the antitumour activity of biologically active compounds from the hemolymph of marine snail *Rapana venosa* and from the mucus of garden snail *Helix aspersa* against the two human cancer cell lines: MDA-MB-231 (triple-negative breast cancer) and A549 (models for the study of lung cancer, p53 positive). Using ultrafiltration on Millipore membrane (100 kDa, 50 kDa and 20 kDa), the hemolymph from *R. venosa* and the mucus from *H. aspersa* were separated into several fractions. The native molecule of *R. venosa* hemocyanin (RvH) and its two isoforms RvH1 and RvH2 were isolated from fraction with Mw above 100 kDa applying different methods such as ultracentrifugation, dialysis and anion-exchange chromatography on FPLC. Reduced cell viability for both tested tumour cell lines is observed after treatment with low concentrations of two mucus fractions from *H. aspersa* with Mw above 20 kDa and above 50 kDa and two isoforms of *R. venosa* hemocyanin (RvH1 and RvH2). The highest antiproliferative effect was established for the fraction with Mw between 50-100 kDa, isolated from hemolymph of *R. venosa*. Chemotherapy drug cis-Platin was tested alone and in combination with the active fractions from the hemolymph of *R. venosa* and the mucus of *H. aspersa* to find synergistic effect of action against the tumor cell lines. The results revealed that the synergistic antiproliferative effect is highest when using the active fraction between 50-100 kDa from hemolymph of *R. venosa* in combination with cis Pt for treatment of MDA-MB-231.

These results may be considered as important information for further investigations of anticancer activity of novel bioactive compounds from hemolymph of marine snail *R. venosa*.

Keywords: *marine snail Rapana venosa, snail Helix aspersa, hemocyanin, anti-tumor activity*

ACKNOWLEDGEMENTS

This work was supported by the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM № 658/14.09.2018 funded by the Bulgarian Ministry of Education and Science.

P4. ANTIOXIDANT AND ANTIFUNGAL PROPERTIES OF MUCUS FROM GARDEN SNAIL CORNU ASPERSUM

Assist. Asya Daskalova¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Assoc. Prof. Dr. Aleksandar Dolashki¹, Assoc. Prof. Dr. Radoslav Abrashev², Assist. Prof. Dr. Nedelina Kostadinova², Assoc. Prof. Dr. Ekaterina Krumova², Prof. DSc. Maria Angelova², Prof. DSc. Pavlina Dolashka¹

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Different species in the phylum Mollusca including garden snails are of increasing interest as a source of novel bioactive compounds. The mucus of garden snail *Cornu aspersum* is a complex mixture of bioactive compounds with potential pharmacological application. The crude extract was separated into several fractions by ultrafiltration on Millipore membrane filters from 10 kDa, 30 kDa, and 100 kDa. The obtained fractions were tested for antifungal activity against *Candida membranifaciens*, *Aspergillus fumigatus* and *Aspergillus niger*.

The total antioxidant potential of the tested fractions was assessed by the DPPH and ABTS radical scavenging activity methods and the nitroblue tetrazolium (NBT) reduction assay; superoxide dismutase (SOD) activity was evaluated as well. Using de novo sequencing (MALDI-MS/MS analysis) we identified the primary structures of above 12 novel antimicrobial peptides with molecular mass between 1-5 kDa. Most of them contain high level of glycine, proline, tryptophan and valine residues which are typical for peptides with antimicrobial activity. The peptides exhibited fungicidal and fungistatic activity agents *Candida membranifaciens*, *Aspergillus fumigatus* and *Aspergillus niger*. The fraction with molecule mass below 10 kDa showed the highest SOD activity. It is possible that the detected SOD value represents the so called "SOD-like" superoxide scavenging. The results from the antioxidant screenings of *C. aspersum* mucus and its fractions show that this naturally derived product has a good potential to counteract the formation of oxygen radicals.

Keywords: *C. aspersum*, antimicrobial peptides, superoxide radicals, antioxidant, antifungal activity

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P5. ANALYSIS OF PROTEINS IN NATURAL EXTRACTS

Dimitar Kaynarov¹, Assist. Asya Daskalova¹, Assist. Ventseslav Atanasov¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Assoc. Prof. Dr. Aleksandar Dolashki¹, Prof. DSc. Pavlina Dolashka¹

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Extracts and different biologically active compounds from several organisms were purified and analyzed either by one-dimensional or two-dimensional sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) with the target to find a mechanism of action for their antitumoral and antimicrobial effects. Hemolymph and mucus of *Cornu aspersum*, *Helix lucorum* and *Rapana venosa* are covered. Information about the molecular weight (MW), the amount and the isoelectric point (pI) of several protein spots were determined by using electrophoresis analysis programs, which are further investigated.

Keywords: SDS-PAGE, natural extracts, protein analysis software

ACKNOWLEDGEMENTS

This work was supported by the National Research Programme "Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)" approved by DCM № 658/14.09.2018 funded by the Bulgarian Ministry of Education and Science.

P6. A ¹H NMR AND MS BASED STUDY OF METABOLITES PROFILING OF GARDEN SNAIL *HELIX ASPERSA* MUCUS

Prof. Dr. Nikolay G. Vassilev¹, Prof. DSc. Svetlana D. Simova¹, Assist. Prof. Dr. Miroslav Dangalov¹, Assoc. Prof. Dr. Lyudmila Velkova¹, Assist. Venceslav Atanasov¹, Assoc. Prof. Dr. Aleksandar Dolashki¹ and Prof. DSc. Pavlinka Dolashka¹

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Metabolic profiling based on ¹H Nuclear Magnetic Resonance (NMR) spectroscopy was applied with the aim to investigate the functional role of the metabolites in lyophilized mucus from garden snail

Helix aspersa. Twenty metabolites were unambiguously identified by ¹H, 1D TOCSY, 2D J-resolved, 2D COSY and 2D HSQC NMR spectra with water suppression. The metabolic profiles of two fractions with low molecular weight (Mw <1 kDa and Mw <3 kDa) are very similar. Metabolites with known antioxidant, antibacterial and antimicrobial activity have been detected by NMR metabolic analysis of mucus samples from *Helix aspersa*. Some of them were confirmed by mass spectrometric analysis. The primary structure of several peptides was identified in low molecular weight fractions (Mw <1 kDa) by tandem mass spectrometry.

Keywords: *mucus, garden snail Helix aspersa, ¹H NMR, Metabolites, mass spectrometry*

ACKNOWLEDGEMENTS

This research was funded by the Bulgarian Ministry of Education and Science through the National Scientific Program “Innovative Low-Toxic Biologically Active Means for Precision Medicine” BioActiveMed, grant number DOI-217/30.11.2018 and by the Bulgarian National Science Fund, grant number DH01/14/19.12.2016.

P7. OREGANO OIL ENCAPSULATED IN CHITOSAN-ALGINATE NANOPARTICLES INHIBITS THE FORMATION OF BACTERIAL BIOFILMS

Assist. Prof. Dr. Lyudmila Dimitrova¹, Assist. Prof. Dr. Maya M. Zaharieva¹, Prof. Dr. Krassimira Yoncheva², Assoc. Prof. Dr. Niko Benbassat², Assoc. Prof. Dr. Alexander Kroumov¹, Assoc. Prof. Dr. Ivanka Spassova³, Prof. Dr. Daniela Kovacheva³, Prof. DSc. Hristo M. Najdenski¹

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Aim of the study is to evaluate the inhibitory effects of oregano oil extracted from *Oreganum vulgare* and encapsulated in chitosan-alginate nanoparticles on the biofilm formation of a panel of pathogenic bacterial strains. In parallel, the general metabolic activity of the treated bacteria was measured and its correlation to the biofilm inhibition capacity was analysed. The biofilm reduction and the bacterial metabolic activity were evaluated by using the protocol of Stepanovich¹ and the MTT test, respectively. The metabolic activity was calculated with the mathematical software MAPLE. The study was performed on four Gram-positive and three Gram-negative bacterial strains. The skin irritation effects of the nano-delivery system was evaluated using ISO10933-10:2010. Pure oregano oil and encapsulated oregano oil inhibited the respiratory activity and the biofilm formation of the treated bacterial strains in concentrations significantly lower than the determined MICs excluding the strains *Streptococcus pyogenes* and *Enterococcus faecalis*. The median inhibitory concentrations calculated for the encapsulated oregano oil were significantly lower than the values of the pure oil. The latter inhibited to a greater extent the biofilm formation of all tested strains. Both forms of oregano oil did not cause skin irritation in albino rabbits. The encapsulated oregano oil is characterized by improved thermal stability and solubility. It exhibited strong inhibitory effects on the metabolic activity and the biofilm formation of the tested pathogenic bacterial strains and is perspective for future development as food additive or preservative in the food industry.

Keywords: *oregano oil, biopolymers, antimicrobial activity, biofilm*

ACKNOWLEDGEMENTS

This study was supported by the National Scientific Program “Innovative low-toxic biologically active agents for precision medicine (BioActiveMed)” (Decision of the Council of Ministers of Bulgaria No. 658/14.09.2018).

P8. *GENTIANA CRUCIATA* L. - SOURCE OF NEW BIOLOGICALLY ACTIVE TRITERPENE FRACTIONS

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Gentiana cruciata is widespread in Europe and Western Asia. The species has been used as appetite stimulants, tonics, aromatic bitters, and for the treatment of gastrointestinal disorders. Bitter constituents belonging to the class of secoiridoid glycosides are typical chemical metabolites for the *Gentiana* plants but in some *Gentiana* species have been identified triterpenes which are also

bioactive compounds with important pharmacological applications.

To the best of our knowledge, no investigations have been reported on the triterpenoid fractions of *Gentiana cruciata*. The present study deals with the identification of nine pentacyclic triterpenes: β -amyrin, α -amyrin, erythrodiol and uvaol, triterpene acids: oleanolic, ursolic, formyl oleanolic and formyl ursolic acids, and methyl ester of ursolic acid. Two of them-3-formyl ursolic acid and 3-formyl oleanolic acid, are described for the first time for the genus *Gentiana*. It is known that pentacyclic triterpenes as ursolic and oleanolic acid have many pharmacological effects including the antioxidant, anti-inflammatory, antibacterial, and antifungal properties. The obtain data could be of use in regard to wide application of the *Gentiana* species in the traditional and complementary medicine.

The triterpenoid profile of *Gentiana cruciata* was determined by GC-MS. Nine pentacyclic triterpenoids corresponding to the ursane and oleanane type have been identified. Their structures have been established by mass spectral fragmentation of TMSi ether derivatives. As a result, 3-formyl ursolic and 3-formyl oleanolic acids were reported for the first time in genus *Gentiana*.

Keywords: *Gentiana cruciata* L., triterpenoids, GC-MS.

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P9. MICELLAR CURCUMIN ENHANCES THE ANTIBACTERIAL ACTIVITY OF THE ALKYLPHOSPHOCHOLINE ERUFOSINE

Assist. Prof. Dr. Maya Zaharieva¹, Assist. Prof. Dr. Lyudmila Dimitrova¹, Assist. Prof. Dr. Alexander Kroumov¹, Assist. Dr. Yana Ilieva¹, Assist. Prof. Dr. Krassimira Yoncheva², Dr. Martin Berger³, Prof. DSc. Spiro Konstantinov², Prof. DSc. Hristo Najdenski¹

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Aim of the study is to evaluate the capacity of micellar curcumin to enhance the activity of the alkylphosphocholine erufosine against methicillin sensitive or resistant pathogenic bacteria from the species *Staphylococcus aureus*. Minimal bactericidal and inhibitory concentrations, metabolic activity and biofilm reduction were determined by ISO 20776-1:2006(E), CFU enumeration, MTT assay and the test of Stepanovic¹, respectively. The metabolic activity was calculated with the mathematical software MAPLE. Curcumin was incorporated in three types of copolymeric micelles: (1) Pluronic P123 (MCRM-P123); (2) mixture of Pluronic P123 and Pluronic F127 (MCRM-P123/F127); (3) methoxy poly(ethylene glycol)-block-poly(ϵ -caprolactone) ensuring prolonged release (MCRM-mPEG-PLC). Rationally selected combinations of micellar curcumin and erufosine significantly increased the antibacterial efficacy of erufosine at concentrations lower than those

determined by single treatment. MCRM-P123/F127 potentiated the anti-staphylococcal activity of erufosine in ratio 1:1, whereas MCRM-mPEG-PLC lead to full eradication of staphylococcal biofilms when combined with erufosine in concentration of 80 μ M and ratio 1:4 [ERF:MCRM]. The micellar formulations with curcumin used in this study can be successfully used for enhancement of the anti-staphylococcal activity of erufosine and prevention of biofilm formation which could be especially beneficial for treatment of malignant diseases accompanied by staphylococcal infections.

Reference: ¹Stepanovic, S., D. Vukovic, et al. (2000). J Microbiol Methods 40(2): 175-179.

Keywords: *curcumin, copolymeric micelles, antibacterial activity, drug combinations*

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P10. PROTECTIVE EFFECT OF MUCUS FROM *HELIX ASPERSA* ON ETHANOL INDUCED GASTRIC ULCERS IN MICE

Assoc. Prof. Lubomir Petrov², Prof. Albena Alexandrova^{1,2}, Assist. Prof. Elina Tsvetanova¹, Dr. Almira Georgieva¹, Prof. DSc. Pavlina Dolashka³, Assoc. Prof. Dr. Lyudmila Velkova³, Assoc. Prof. Dr. Alexander Dolashki³, Assist. Venceslav Atanasov³

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In recent years, there has been an increased interest in research into the biological effects of natural compounds with preventative or therapeutic potential for various pathological conditions. A number of studies have shown that the mucus of snails is a multi-component mixture comprising various substances with antibacterial, antiviral, antifungal and antioxidant activity (Dolashka et al., 2015). Given the involvement of oxidative stress in the pathogenesis of gastric ulcer, as well as the wide range of bioactive compounds of *Helix aspersa* mucus, incl. skin regeneration potential (Trapella et al., 2018) and antioxidant capacity (Kostadinova et al, 2018), this study aimed to investigate the possible protective effect of *Helix aspersa* mucus on ethanol induced gastric ulcers in mice and elucidate its efficacy mechanism in terms of oxidative stress measures. Male Albino mice were divided in three groups: Control (untreated mice), Ethanol (mice with ethanol induced gastric ulcers) and Mucus+Ethanol (mice pretreated with snail mucus and ethanol induced gastric ulcers). The mice of Mucus+Ethanol group were pretreated with snail mucus (10 ml/kg, per os) one hour before ulcer induction. The gastric ulcers were induced by administration of 96% ethanol (10 ml/kg, per os). The effect of mucus in this model was evaluated by determining the gastric ulcers index (GUI), lipid peroxidation (LPO) and glutathione concentration (GSH) in stomach homogenates and the activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in stomach postmitochondrial cytosolic fraction. The administration of ethanol to mice caused grave lesions with large hemorrhagic necrosis of gastric mucosa and significant decrease of GSH levels and activities of SOD, CAT and GPX. In the animals with pre-administration of snail mucus were observed: only a small number of hemorrhagic fields; significantly reduced GUI compared to ethanol group (4.7% vs 17.3% respectively) ($P < 0.001$); protection percentage calculated - 73%; significant recovery of mucosal GSH level and activity of GR; rise in the activity of the other tested enzymes. The positive effect of snail mucus in this model of gastric injury probably is a result of the complex action of many factors. Despite the fact that the exact mechanism is not completely clarified, the results obtained give us reason to believe that snail mucus can serve as a good tool for treatment or concomitant treatment of gastric ulcer.

Key words: *Gastric ulcers, Helix aspersa mucus, Oxidative stress*

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P11. NATIONAL RESEARCH PROGRAMME “INNOVATIVE LOW-TOXIC BIOACTIVE SYSTEMS FOR PRECISION MEDICINE (BioActiveMed)”

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The National Research Programme “*Innovative Low-Toxic Bioactive Systems for Precision Medicine (BioActiveMed)*” is focused on the development of new low toxic bioactive substances and systems containing extracts of natural sources (of plant or animal origin) from Bulgaria for the prevention and support of the therapy of certain diseases. The first step is the isolation and purification of bioactive substances of plant and animal origin, their appropriate incorporation in suitable innovative systems and development of new methodologies for their characterization and determination of quality and applicability as new products in personalized and preventative medicine. One of the planned long-term applications is the implementation of new innovative healthy and safe food supplements and cosmetics with a preventive potential for personalized medicine.

The research has been carried out with the participation of leading scientists with proven scientific capacity, as well as with the active participation of young scientists and PhD students from the Consortium formed. Web page of the Programme is: www.bioactivemed-nrp.com.

Keywords: *extracts of natural sources, bioactive compounds, innovative systems, precision medicine, food supplements and cosmetics*

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P12. CHARACTERISTICS OF NOVEL PEPTIDES OF *CORNU ASPERSUM* MUCUS WITH ANTIBACTERIAL ACTIVITY

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Natural compounds have a great potential for use in new antimicrobial drugs, because some of them have a pronounced cytotoxicity to a number of multi-drug resistant bacteria. The aim of the present study is to isolate and identify new antimicrobial peptides from the mucus of garden snail *Cornu aspersum* and to analyze their antibiotic activity against *Staphylococcus aureus* (Gram⁺) and *Escherichia coli* (Gram⁻) bacteria.

The results show that only peptide fraction below 10 kDa manifest a strong antibacterial activity against the selected pathogens. Analysis of the morphology of the cells treated with peptide fraction shows a strong deformation. Craters, wrinkles and bubbles are visible on the surface of the bacteria. To explain observe effect we identified the primary structures of many novel peptides and

glycopeptides in active fraction, using tandem mass spectrometry (MALDI-MS/MS and Q-trap LC/MS/MS analysis). We have determined amino acid sequences and carbohydrate structures of 6 *N*-glycopeptides from high-mannose and complex type. Some of them contain terminal MeHex residue and modification to inner core by xylose. The presence $\beta(1,2)$ -xylose linked to the core mannose is a highly immunogenic epitope for mammalian species. The *O*-methylated glycans has been found only in worms and molluscs, more frequently present in some species of bacteria, fungi and plants, but not in mammals. *O*-Methylated glycans probably play a role in some recognition events, as well as in human infections and diseases, but mechanism is still unknown.

Our results can be considered as basic information for further investigations on antimicrobial peptides from *C. aspersum* and their potential biomedical applications.

Keywords: *C. aspersum*, mucus, tandem mass spectrometry, antimicrobial peptides, glycopeptides, *Staphylococcus aureus*, *Escherichia coli*.

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P13. CONFORMATIONAL STABILITY OF HEMOCYANIN ISOLATED FROM SNAILS *HELIX LUCORUM*

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Hemocyanins (Hcs) are extremely large extracellular oxygen transport glycoproteins isolated from different arthropod and mollusc species. Various aspects of biomedical applications of molluscan Hcs, associated with their immunogenic properties and antitumor activity, promoted us to perform structural studies on a representative of these proteins.

The conformational stability of the Hc, purified from the hemolymph of garden snails *Helix lucorum* (HIH), was studied using various biophysical methods: transmission electron microscopy, far-UV circular dichroism and differential scanning calorimetry.

Higher concentrations of Ca^{2+} and Mg^{2+} ions and pH values 6.5 – 8.0 promote the stability of the protein molecule. One transition, with an apparent transition temperature (T_m) at 82.3 °C, was detected in the heat capacity curve of HIH in 50 mM Tris-HCl buffer, pH 7.2, at a heating rate of 1.0 °C min⁻¹. The calorimetrically observed thermal transition correlates well with the unfolding transition monitored by CD measurements. The two-state kinetic model was used to analyse the process of irreversible thermal denaturation of HIH, E_a 451 ± 4 kJ mol⁻¹ was calculated.

The data obtained will serve as a basis for the development of stable Hc preparations and will facilitate further study of the properties and potential biomedical applications of this oxygen-carrying protein.

Keywords: hemocyanins, arthropod, mollusc, immunogenic properties, antitumor activity.

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P14. ANTIFUNGAL ACTIVITY OF THE HEMOLYMPH ISOLATED FROM MOLLUSKS AND ARTHROPODS

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Antibacterial and antifungal activity of bioactive compounds is a new direction of scientific searching. Mollusks have been known as a source of novel natural products with antibacterial activity. But the compounds exhibiting antifungal effect are very rare found. At the same time different species, belonging to genera *Aspergillus*, *Mucor*, *Penicillium*, *Cladosporium*, *Fusarium*, and *Alternaria* have been reported as etiological agents of respiratory disorders, onychomycosis, asthma with fungal sensitization, etc.

The aim of the present study was to investigate the fungicidal and fungistatic activity of hemolymph fractions isolated from *Rapana venosa*, *Carcinus aestuarii*, and *Helix lucorum* against strains belonging to above mentioned genera.

The hemolymph from *R. venosa*, *C. aestuarii*, and *H. lucorum* was collected and purified using different membrane techniques. The crude extract was separated using Millipore filters by ultrafiltration into different fractions. Ten hemolymph fractions were tested for their antifungal activity against fungi belonging to 5 genera: *Mucor hiemalis*, *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium tubingensis*, and *Alternaria solani*.

Clear inhibitory effect was established after treatment with dissociated hemocyanin from *C. aestuarii*, hemolymph fraction < 100 kDa from *R. venosa* and fraction < 100 kDa from *H. lucorum*. The microplate assay confirmed antifungal activity of mentioned fractions. Antifungal activity was species dependent. These three fractions demonstrated strong fungicidal effect for a long time, for 7 days in concentration 12 µL/well.

Dissociated hemocyanin from *C. aestuarii* and peptide fraction < 100 kDa from *R. venosa* could be perspective compounds for the next investigations.

Key words: mollusks, arthropods, hemolymph, antifungal activity, *Rapana venosa*, *Helix lucorum*, *Carcinus aestuarii*

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