VI Simpozijum Srpskog udruženja za proteomiku (SePA)
"Razvoj i primena novih metoda proteomike"

Rektorat Univerziteta u Kragujevcu
2. jun 2023. godine

Book of abstracts

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VI Simpozijum Srpskog udruženja za proteomiku "Razvoj i primena novih metoda proteomike"

13:00 **Prof. dr Marija Stanić** - dekan PMF Kragujevac, **Prof. dr Nevena Đukić**, PMF- Kragujevac, otvaranje VI SePA simpozijuma.

13:10 **Dr Lidija Izrael – Živković**, "Proteome changes of the model bacteria *Pseudomonas aeruginosa* san ai exposed to nanoceria", Institut za hemiju, Medicinski fakultet, Univerzitet u Beogradu, Višegradska 26, Beograd, Srbija

13:30 **Dr Ana Medić**, "Flexibility of carbon catabolic pathways in *Pseudomonas aeruginosa* san ai during the biodegradation of toxic organic compounds- a multiomics approach", Institut za hemiju, Medicinski fakultet, Univerzitet u Beogradu, Višegradska 26, Beograd, Srbija

13:50 **Dr Katarina Smiljanić**, "Alterations in proteomic profiles of lung epithelial cell line BEAS 2B upon treatments with electronic cigarettes liquids and pure nicotine", Univerzitet u Beogradu – Hemijski fakultet, Studentski trg 12-16, Beograd, Srbija

14:10 **Dr Nataša Avramović**, "Application of NMR spectroscopy in metabolomics", Institut za hemiju, Medicinski fakultet, Univerzitet u Beogradu, Višegradska 26, Beograd, Srbija

14:30 **Dr Romana Masnikosa**, "Plasma profile of inflammatory mediators in NHL patients", Institut za nuklearne nauke „Vinča", Laboratorija za fizičku hemiju, Mike Petrovića Alasa 12-14 11351 Vinča, Beograd, Srbija

14:50 Pauza: Poster sekcija

15:10 **Dr Marko Živanović**, "Scaffolds for *in vivo* wound healing", Institut za informacione tehnologije Kragujevac, Jovana Cvijića bb, 34000 Kragujevac.

15:30 **Dr Milan Mladenović**, "Computational Approaches in Modulating the Estrogen Receptor α; Signaling: A Pathway for Breast Cancer Cure Discovery?", Univerzitet u Kragujevcu, Prirodno – Matematički fakultet, Institut za Hemiju, Radoja Domanovića 12, Kragujevac, Srbija

15:50 **Dr Milena Milutinović**, „The impact of natural products on the expression of apoptosis and biotransformation-related genes and proteins in immortalized carcinoma cell lines" Univerzitet u Kragujevcu, Prirodno – Matematički fakultet, Institut za Biologiju i Ekologiju, Radoja Domanovića 12, Kragujevac, Srbija

16:10 **Dr Maja Krstić Ristivojević,**"Identification of isoforms of shellfish tropomyosin" Univerzitet u Beogradu – Hemijski fakultet, Studentski trg 12-16, Beograd, Srbija

16:30 **Dr Nikola Gligorijević,** "Biocorona formation of hen proteins onto the surface of polystyrene and polyethylene terephthalate", Univerzitet u Beogradu – Hemijski fakultet, Studentski trg 12-16, Beograd, Srbija

16:50 **Dr Dragana Filipović**, "Chronic fluoxetine treatment of socially isolated rats modulates prefrontal cortex proteome", Institut za nuklearne nauke „Vinča", Laboratorija za molekularnu biologiju i endokrinologiju, Mike Petrovića Alasa 12-14 11351 Vinča, Beograd, Srbija

17:10 Analysis doo

17:30 Diskusija i zatvaranje skupa

18:00 Godišnja skupština društva

Proteome changes of the model bacteria *Pseudomonas aeruginosa* san ai exposed to nanoceria

Lidija Izrael Živković [1*]

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Nanoceria (NC) have been used as carrier for targeted drug and gene delivery, as an antidiabetic and antibacterial agent, and for tissue engineering. An environmental isolate of the Gram-negative bacterium *Pseudomonas aeruginosa* san ai was used as a model organism[1] for the study and deeper understanding of the impact of NC. Total proteome was monitored, coupled with changes in the profile of targeted secondary metabolites. Comparative proteomic analysis was performed using a comprehensive, high-throughput bioanalytical nLC-MS/MS platform coupled with bioinformatics.

Increased production of proteins related to redox homeostasis, amino acid biosynthesis and lipid catabolism upon NC exposure was observed. Most of the downregulated proteins from outer cellular structures are ABC transporters. As evidence of changes in redox homeostasis, increased production of pyocyanin, a key redox molecule, and pyoverdine, a siderophore responsible for iron homeostasis was detected.[2]

In the presence of sublethal concentrations of NC, the bacteria can become even more potent due to its enhanced production of virulence factors.

Acknowledgements

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Flexibility of carbon catabolic pathways in *Pseudomonas aeruginosa* san ai during the biodegradation of toxic organic compounds- a multiomics approach

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Environmental contamination caused by petroleum hydrocarbons and phenolic compounds poses a significant challenge in the era of industrialization and advanced technology. *Pseudomonas* species, found in almost all hydrocarbon-contaminated areas, play a crucial role in the biodegradation of these pollutants. The genus *Pseudomonas* has the remarkable ability to degrade various hydrocarbons and phenolic compounds, utilizing them as the sole source of carbon [1]. *Pseudomonas* employs the plasticity of its carbon metabolism as an adaptive strategy to survive exposure to toxic organic compounds [2,3]. Understanding the mechanisms by which *Pseudomonas* degrades pollutants is essential for developing novel environmental remediation strategies. *Pseudomonas* utilizes various upper and lower metabolic pathways to transform and degrade hydrocarbons, phenolic compounds, and petroleum hydrocarbons.

The emergence of newly developed analytical omics platforms provides enormous potential not only for improving our understanding of processes at the molecular level, but also for investigating and monitoring the complex biodegradation processes mediated by *Pseudomonas*. Additionally, there is a growing interest in applying the aromatic metabolic pathways of *Pseudomonas* in biotechnological endeavors such as the bioremediation of crude oil-polluted environments, the biovalorization of lignin for the production of bioplastics and biofuels, as well as *Pseudomonas*-assisted phytoremediation.

Acknowledgements

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Proteomic and protein modification profiling of lung cells BEAS 2B upon different electronic cigarette vapour treatments

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Although e-cigarette are still considered a safer alternative to traditional cigarettes, a growing body of evidence points to their harmful effects on a range of cellular processes. In this study lung BEAS 2B epithelial cells were treated for 24 h with sub-cytotoxic concentration[1] of e-cigarette vapour, with and without (w/o) nicotine and (w/o) flavor. The comprehensive proteome analysis was performed via high resolution mass spectrometry based proteomics (Orbitrap Exploris 240, Thermo Scientific, USA) and relative, label free quantification of protein expression and their post-translational and chemical modifications by PEAKS X Pro Studio (BSI Ltd, Ontario, Canada). E-cigarette liquids induced significant depletion in total number of proteins and impairment of mitochondrial function in treated cells. Increased presence of post-translational modifications, including environmentally-driven toxic&harmful, and those classified as direct oxidative modifications, were observed especially in combined nicotine+flavour treatment and flavour treatment without nicotine, beside control, pure nicotine and base liquid treatments. There is a need to study further biological effects of e-cigarettes in more details, given their widespread use.

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Application of NMR spectroscopy in metabolomics

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Metabolomics contributes to comprehensive qualitative and quantitative low-molecular-weight metabolites' (< 2 kDa) analyses in cells, tissues, and body fluids, and considers the alteration in metabolites as result of modified biochemical pathways related to the pathogenesis of disease. Throughout comparison among individuals from diseased and healthy groups, metabolomics provides determination of the objective biomarkers.[1,2] In addition to gas chromatography coupled to tandem mass spectrometry (GC-MS/MS), and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), nuclear magnetic resonance spectroscopy (NMR) is the main analytic technique to explore metabolomics. Although NMR-based metabolomics has the disadvantage of lower sensitivity compared to MS-based methods, it has significant advantages including simple preparation of samples, high reproducibility, determination of either known or unknown molecule structures, and the possibility to analyze *in vivo* and *ex vivo* samples, which is especially important for clinical research. Recently, a panel of potential biomarkers was explored and identified in biological fluids of patients with cancer and psychiatric diseases by applying ^{1}H-NMR-based metabolomics.[1,2] Reported studies demonstrated that diverse metabolites are correlated with the altered biochemical pathways, including mitochondrial/energy metabolism, oxidative stress, amino acid metabolism, and lipid metabolism.

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Plasma Profile of Inflamatory Mediators in NHL Patients

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Both cancer and inflammation are almost invariably accompanied by lipidome dysregulation. Hence, various lipid species have been reported as candidate markers for many solid tumours[1-3]. However, neither the global lipidome nor sub-lipidome of inflammatory pathways in Non-Hodgkin lymphoma (NHL) has been studied. In order to fill this gap and shed light on the inflammatory pathways accompanying NHL, we designed a targeted liquid chromatography – Multiple Reaction Monitoring of bioactive lipids/lipid mediators in plasma of female patients with Diffuse Large B-cell Lymphoma (DLBCL), the most often type of NHL. We chose to quantify lipids known or hypothesized to be involved in inflammation and cancer progression along with their membrane precursors. In a pilot study encompassing plasma samples from 17 DLBCL patients and 21 BMI-matched controls, we analysed levels of pro-inlammatory arachidonic acid (AA)-derived oxylipins, focusing on lipoxygenase (LOX) and cytochrome P450 monooxygenase products: hydroxyeicosatetraenoic acids (HETEs) and dihydroxyeicosatrienoic acids; several AA-containing phospholipids (PLs); specific lysophospholipid subclasses; sphingomyelins (SMs), sphingosine 1-phosphate (S1P) and polyunsaturated fatty acids. Data were subjected to classical statistics and multivariate unsupervised and supervised machine learning (ML) algorithms. The DLBCL status was profoundly associated with altered S1P, SM 34:1, SM 36:1 and phosphatidylinositol PI 34:1 abundance. On the other hand, eicosanoids 12(S)-HETE, 15(S)-HETE and thromboxane B2 were major lipid species discriminating between DLBCL and healthy status, as well as lysophosphatidylinositol LPI 20:4. The correlations between lipid species varied considerably between the cancer and controls, reflecting significant changes in lipid metabolic and/or signalling pathways, particularly those within LOX pathway and cell membrane PL remodelling. We suggest S1P, SM 36:1, SM 34:1 and PI 34:1 may beviewed as lipid signatures of DLBCL. Furthermore, these four lipid species could serve asa basis for the prospective validation in larger DLBCL/NHL clinical studies. As far as we know, this is the first plasma lipid profiling in DLBCL/NHL and, as such, brings new knowledge on the metabolic basis of inflammation in this cancer. The added value of our plasma lipid profiling in DLBCL is a deeper understanding of particulate lipid dysregulations in this tumour.

Acknowledgements

LC-MRM analyses were performed at the Clinical Lipidomics Unit of the Institute of Physiological Chemistry, University Medical Center Mainz, Germany, under the supervision of dr Laura Bindila, head of the Unit. The authors thank Dr Bindila for SOPs, providing access to AB Sciex Q-TRAP 5500 mass spectrometer, technical advice and helpful discussions. The authors thank dr Julia Postand dr Raissa Lerner for help with eicosanoids and phospholipid data analyses, respectively, and also Claudia Schwitter for the technical support.

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Scaffolds for *in vivo* wound healing

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We synthesized 125 different solutions of polymers, including polycaprolactone, polyethylene glycol, and their combinations, using solvents chloroform and dimethylformamide, at various concentration ratios. These solutions were electrospun into microfiber meshes to create scaffolds for *in vivo* wound healing applications. We analyzed the materials obtained from this process using microscopic techniques. Our artificial intelligence (AI) model used the chemical composition and electrospinning parameters as input to predict the optimal solution for scaffold production based on fiber diameter measurements. After comparing the AI model's predictions with experimental data, we selected the most suitable scaffold for further *in vitro* analyses. To enhance its efficacy in wound healing, we modified the chosen scaffold by adding antibiotics. The results revealed three important findings: 1) AI is highly valuable in material and biomedical sciences; 2) our combined methodology serves as an effective initial screening approach for biomedical materials; 3) the electrospun scaffolds with antibiotics displayed remarkable performance in stimulating angiogenesis and treating wounds *in vivo*.

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Computational Approaches in Modulating the Estrogen Receptor α Signaling: A Pathway for Breast Cancer Cure Discovery

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The estrogen receptor α (ERα) represents a 17β-estradiol inducible transcriptional regulator that initiates the RNA polymerase II-dependent transcriptional machinery, pointed for breast cancer (BC) development *via* either genomic direct or genomic indirect (*i.e.,* tethered) pathway. To develop innovative ligands, structure-based (SB) 3-D QSAR, ComBinE, and 3-D Pharmacophore studies have been undertaken from experimentally resolved partial agonists, SERMs, and SERDs within either wild-type or mutated ERα receptors. SB and ligand-based (LB) alignments gave rules to align the untested compounds. The protocols led to the development of **3DQ**s, **CBE**s, and **3DPQ**s compounds, further synthesized and submitted to either *in vitro* or *in vivo* assessments, upon which new leads were revealed as candidates for clinical trials.[1-2]

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The impact of natural products on the expression of apoptosis and biotransformation-related genes and proteins in immortalized carcinoma cell lines

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Many of commercially used anticancer drugs have been obtained from the nature, isolated, purified, modified, or synthesized according to natural compound as a model. The large number of compounds from nature (extracts of plants and their secondary metabolites, animal products and biotoxins and their constituents) have been testing in our experimental conditions to determine their anticancer potential, including their cytotoxicity, proapoptotic activity, affected gene and protein biomarkers of apoptosis as potential targets, antiinvasive, antimigratory, antiangiogenic activity, etc [1,2]. One of the leading problems and failures in anticancer therapy is development of drug resistance, which is frequently associated with the overexpression of biotransformation enzymes and ABC transporters on cell membrane. Natural compounds which belong to Fourth Generation Inhibitors of multi drug resistance have potential to become more successful than previously developed modulators and deserve future detailed investigations.

Acknowledgements

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Identification of isoforms of shelfish tropomyosin

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From over 10 kg in 1960 to over 20 kg in 2014, the yearly per capita consumption of marine products has increased significantly during the past 50 years. In many nations, especially those with poor overall protein intake, seafood protein is a crucial component of the diet[1]. However, as defined by the European Community shellfish protein tropomyosin (TPM) is one of the major allergens and major causes of anaphylaxis[2]. Although TPM originating from vertebrates is not considered as an allergen there is evidence that several fish tropomyosin can be allergenic[3]. TPM protein is organized of two parallel alpha-helical molecules which are wound around each other forming a coiled-coil structure[2]. Although the degree of similarity between TPM molecules is high, their allergenic potency is different. Scientists putting a lot of effort into identifying and sequencing tropomyosin isoforms since this information probably explains the intriguing nature of the TPM molecule. This is a very challenging task given that the differences in mass and pI values between TPM isoforms are discrete.

TPM was isolated from mussels (*Mytilus galloprovincialis*), and clams (*Venerupis philippinarum*) according to the protocol developed within/and for purposes of the IMPTOX research project. The obtained "in-house" TPM proteins were resolved using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Isoelectric focusing was performed on rehydrated Immobiline strips IPG3-5.6NL 13 cm in Ettan IPGPhor 3 device. The second dimension was carried out on 12% PAA gels. Upon protein bands excision to prevent TPM interchain disulfide cross-linking reduction and alkylation of cysteine was performed. The samples were digested with proteomics-grade trypsin in a 1:50 enzyme-to-substrate ratio overnight at 37 °C. The obtained peptides were chromatographically separated using the EASY-nLC II system and analyzed using Orbitrap Exploris 240 mass spectrometer.

2D-PAGE resolved two isoforms of mussel TPM and up to eight isoforms of clam TPM. The determined pI value of the dominant isoform of mussel TPM was 4.7 while the discrete band arising from the second TPM isoform was slightly shifted toward acidic pI and smaller molecular mass. The determined pI value of the three most dominant clam TPM isoforms was 4.8, the fourth isoform was slightly shifted toward a more basic pI value and lower protein molecular weight. The rest of the isoforms were slightly shifted toward more acidic pI and at a similar molecular weight as the three dominant isoforms.

The mass spectrometry results were obtained using PEAKS software using *de novo*, database, and SPIDER search. In the MS data evaluation, we tried to implement one of the promising approaches for resolving the protein amino acid sequences proposed by Tran and colleagues[4] that relies on the principle of the Bruijn graph applied to de novo sequences. Unfortunately, this approach didn't lead to the identification of TPM isoform sequences since there was a low abundance of less dominant isoforms. Mass spectrometry results using database search (using a PDB library of all known TPM sequences) in dominant mussel TPM isoform identified 25 peptides which cover 42 % of amino acid (AA) sequence of TPM with NCBI accession P91958. Regarding less abundant mussel TPM isoform 21 peptides were identified which interestingly cover 36 % of the AA sequence of Penaeus monodon, NCBI accession AAX37288. Although up to 8 isoforms of clam TPM were been detected upon 2D-PAGE, 6 bands were successfully excised. In the most dominant clam TPM isoform 35 peptides were identified which cover 61 % of the AA sequence of TPM with NCBI accession BAH10157. Although the number of identified peptides in less present clam TPM isoforms decreased, peptides with different amino acids were discovered in isoforms three and five compared to peptides at the same position in the dominant isoform. Using sequence alignment of identified peptides in dominant and less abundant mussel and clam TPM isoforms it was possible to detect to some extent the substitution of particular AA among isoforms.

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Biocorona formation of hen egg white proteins onto the surface of polystyrene and polyethylene terephthalate

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Ovalbumin (OVA), a main protein of egg white, has characteristic structural fold of a serpin-family of proteins, propensity to fibril formation and stability to digestion. Microplastics (MPs) contaminating our food can interact with food proteins in the food matrix and during digestion. In this study adsorption of OVA to polystyrene (PS) (110 μm and 260 μm), polyethylene terephthalate (PET) (140 μm) MPs were investigated in acidic (pH 3) and neutral (pH 7) conditions. Formations of corona on MPs were investigated using isolated OVA and egg white protein extract comparatively. OVA adsorption depends on MPs size, polymer chemistry and pH, being highest in acidic pH and higher for PS. Adsorption of OVA to PS and PET reaches dynamic equilibrium after 4h resulting in disruption of tertiary structure and formation of hard and soft corona around MPs. Shorter fragments of OVA populate hard corona, while soft corona exclusively consist of full length OVA, albeit in its non-native conformation. The conformational changes resemble those induced by heat treatment with re-arrangement of α-β secondary structures. Structural changes are striking for the OVA in corona around MPs. Soft corona OVA preserves thermal and proteolytic stability, but loses ability to form fibrils upon heating. OVA is abundantly present in corona around MPs also in the presence of other egg white proteins. MPs contaminating food may bind and change structure and functional properties of main egg white protein.

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Chronic fluoxetine treatment of socially isolated rats modulates prefrontal cortex proteome

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Exposure to chronic social isolation (CSIS) and dysfunction of serotonin neurotransmission have been implicated in the etiology of major depressive disorder (MDD). Fluoxetine (Flx) has been widely used to treat MDD, however, its molecular mechanisms of action are not yet defined. Hence, we carried out a comparative label-free proteomic approach to identify sub-proteome changes in the prefrontal cortex (PFC) cytosol, non-synaptic mitochondrial (NSM), and synaptosomal-enriched fractions of adult male Wistar rats following chronic social isolation (CSIS) (6 weeks), a rat model of depression, and/or following Flx treatment in CSIS and control rats (15 mg/mL/day) (lasting 3 weeks of 6-weeks CSIS) using liquid chromatography coupled to tandem mass spectrometry. Our aim was to identify the changes in protein levels that enable the identification of (possible) biochemical pathways and processes of importance for the development of depressive-like behavior and the efficacy of Flx treatments. Behavior was assessed with sucrose preference and forced swim tests. In controls, Flx downregulated the proteins involved in endocytosis and vesicle-mediated transport, while predominantly upregulating proteins involved in the microtubule cytoskeleton, intracellular calcium homeostasis, an enzyme linking the glycolytic pathway to the citric acid cycle in NSM, and exocytosis. CSIS affected the PFC proteome by downregulating the proteins involved in proteasome pathway, glutathione antioxidative system, synaptic vesicle cycle, and endocytosis while upregulating the protein levels of enzymes participating in oxidative phosphorylation 1,2. CSIS compromised mitochondrial membrane integrity, as assessed by cytochrome c levels in the cytosol. Effective Flx treatment in CSIS rats resulted in increased synaptic vesicle dynamic, plasticity, and mitochondrial functionality and a suppression of CSIS-induced impairment of these processes [1,2]. Our data provide the basis for establishing a marker panel for CSIS-induced depression and effective Flx treatment and highlight the role of NSM and synaptosomal proteins involved in various biochemical pathways as novel investigative protein targets.

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Proline accumulation as an adaptive response to heat stress in different wheat cultivars

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High temperature stress is one of the environmental factors that lead to crop yield reduction around the world[1]. As one of the organic osmolytes and protein stabilizer, proline can accumulate in plants in response to stressful environmental conditions [2]. The aim of this research was to determine the concentration of proline in ten winter wheat cultivars under conditions of high temperature stress and to assess the variability of wheat cultivar responses. The correlation of proline accumulation with photosynthetic pigments and yield was also determined. The results showed that there is a statistically significant difference between proline values in moderate and high air temperature conditions when proline accumulation occurs. A significant correlation was found between accumulated proline and yield, especially in wheat cultivars Apač, Talas and Futura. Those cultivars had higher proline values under heat stress and higher yield. Correlation is also found between proline concentration and photosynthetic pigment contents, where wheat variety Apač showed the best adaptive response related to investigated traits and was characterized with lower decrease in photosynthetic pigment content under heat stress. The interrelation of proline with the photosynthetic pigments content, and wheat quality parameters may be important in breeding technologies aimed at improving wheat stress tolerance.

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Antitumor capacity of *Thymus zygis* essential oil in various human cancer cell lines

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Cancer is one of the most persistent diseases with high mortality worldwide. Free radicals are known to be involved in tumor progression, but overproduction may lead to a negative clinical impact on therapy by causing chemoresistance. Additionally, we have examined the possible antitumor mechanisms of *Thymus zygis* essential oil on human colon cancer HCT-116, breast cancer MDA-MB-231 and chronic myelogenous leukemia K562 cell line including cell viability and redox potential. The cells were treated with different concentrations of essential oil (from 1 μg/mL to 200 μg/mL) during 24 h and 72 h. The tested essential oil expressed antiproliferative activity, determined by MTT assay and, as well as dose- and time-dependent increase of nitrites and decreased of $O_2^{\cdot-}$ production. The antiproliferative potential appeared to be dose - and time-dependent, since the obtained results showed that all used concentrations of essential oil exhibited decreased viability of HCT-116, MDA-MB-231 and K562 cells. The stronger antitumor effects have been shown in MDA-MB-231 cells after long-term treatment, especially at the highest applied concentration, where the percentage of viability was reduced for over 53%. The results also showed decreased concentrations of superoxide anion radical in treated d cells, which indicates their significant antioxidative role. Elevated levels of nitrites indicate high levels of nitric oxide (NO) production and suggest its higher bioavailability due to antioxidative environment.

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Effects of novel Pt(IV) complexes on kidney of rats

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Nephrotoxicity is a common consequence of the non-selective action of chemotherapeutics. To overcome the negative effects of chemotherapeutics, new platinum (IV) complexes are synthesized. The aim of this study is to examine the effects of novel synthesized Pt(IV) complexes containing ethyl- and propyl-esters of the ethylenediamine-*N,N'*-di-*S,S*-(2,2'-dibenzyl) acetic acid on the kidneys of Wistar albino rats through the detection of oxidative stress parameters and morphological tissue changes followed by histopathological analysis. The rats were intraperitoneally treated (single dose of 10 mg/kg b.w.) with the tested complexes and the results were compared with the values measured in control animals (i.p. treated with saline). Used complexes significantly decreased the production of O2.- and H2O2, while the level of NO2- and LPO were increased. Additionally, the activity of SOD increased, while the activity of CAT was alleviated. Regarding morphological changes in the kidney tissue, moderate hydrops degeneration, necrosis, atrophy and desquamation of the tubular epithelium cells were observed, while other changes were of mild intensity. These results indicate that nowel Pt(IV) complexes could induce slight nephrotoxicity and are useful for further investigation related to elucidating the mechanism of their action.

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Electrophoretic assessment of recombinant λ-exonuclease production in different *E. coli* strains

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Viral exonucleases play role in many processes essential for genome maintenance, including DNA repair and recombination. Lambda exonuclease (λ -exo), isolated from lambda bacteriophage, hydrolases double-stranded DNA (dsDNA) in the highly processive manner in 5'→3' direction, yielding mononucleotides and single -stranded DNA (ssDNA). This unique enzymatic properties offer several promising biotechnological applications, such as highly sensitive quantification of DNA modifications and single -molecule sequencing. Hence, optimization of the expression conditions is a prerequisite to achieve high-level production of λ-exo. Here we have tested λ -exo expression in five different *E. coli* strains under various temperature regimes in order to establish the optimal conditions for efficient production of recombinant λ-exo. The N-terminally His -tagged λ-exo was successfully expressed in *E.coli* BL21(AI), SHuffle T7, C41(DE3) and C43(DE3) strains in LB broth. Collected aliquots were analysed by SDS-PAGE, followed by CBB staining. Relative yield of target protein bands was determined by densitometry in total cell lysate, as well as in soluble and insoluble cytoplasmatic fractions. We identified *E.coli* BL21(AI), SHuffle T7 and C41(DE3) as good producers of recombinant λ-exo, and upon scaling up, λ -exo was purified from crude cell lysates by metal affinity chromatography in satisfactory yield. Our data suggest that densitometric analysis could serve as a powerful low-cost screening platform for improving recombinant protein expression strategies.

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Densitometric analysis of protein profiles as a tool for DoE decision making for recombinant protein production

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The optimization of expression conditions is a tedious, but necessary procedure for the successful efficient production of recombinant proteins. Design of experiment (DoE) saves time and resources by using statistics to carefully select only a few experimental points (expression conditions) to obtain information about the whole tested range of varied conditions, unlike the most commonly used one-factor-at-a-time (OFAT) method, which requires full imput space screening for optimization. Here, we have optimized the expression conditions of His_6 and His_8-mCerulean3-TEV-α-synuclein production from the pDUET vector, as well as the His_6-tagged protein from the pET-20b vector and expressed into the periplasm. The proteins for which optimization was conducted were expressed in *Escherichia coli* BL21(DE3) and BL21(DE3)pLysS. We have used DoE to plan adequate experimental points for optimization according to a Box-Behnken design, with IPTG concentration, temperature and time variation. After collecting the cultures at given experimental points, they were analyzed by SDS-PAGE and densitometry, then modelled and statistically analysed. The importance of correct sample preparation and gel loads for densitometric analysis is evident according to the obtained results. The largest expression level was obtained from the His_8-tagged protein coded by the pDUET vector in *E. coli* BL21(DE3). We have also used densitometry to analyze expression levels in different culture media.

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