2.EBAT

EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS

26-29 OCTOBER 2019 ANTALYA

ABSTRACT BOOK

ANKARA ÜNİVERSİTESİ 1946

ESKİŞEHİR OSMANGAZİ ÜNİVERSİTESİ 1970

MALATYA TURGUT ÖZAL ÜNİVERSİTESİ 2018

TURKISH CHEMISTS SOCIETY

Türkiye

Sustainability Committee

Prof. Ahmet **ÇOLAK**, Karadeniz Technical University

Prof. Hasan **ÖZDEMİR**, Atatürk University

Prof. Ali **KILINÇ**, Ege University

Prof. Emel **EMREGÜL**, Ankara University

Assoc. Prof. Ali **ALKAN**, Karadeniz Technical University, Chairman of Chemists Society Karad. Branch

Chairman

Prof. Emel **EMREGÜL**, Ankara University

Vice Chairmen

Prof. Ahmet **ÇABUK,** Eskişehir Osmangazi University (Vice Rector)

Prof. İsmet **YILMAZ**, Malatya Turgut Özal University (Vice Rector)

Congress Secretary

Assoc. Prof. Pınar Acar **BOZKURT,** Ankara University

Congress Coordinator

Chem. İkram **CENGİZ**, Chairman of Chemists Society

Advisory Board

Prof. Emel **ARINÇ**, Middle East Technical University (Retired)

Prof. Ural **AKBULUT**, Middle East Technical University (Retired)

Prof. Ö. İrfan **KÜFREVİOĞLU**, Atatürk University

Prof. Şule **PEKYARDIMCI**, Ankara University (Retired)

Prof. Seyhan **TÜKEL**, Çukurova University (Vice Rector)

Prof. Oktay **ARSLAN**, Balıkesir University

Organizing Committee

Prof. Orhan **ATAKOL**, Ankara University

Prof. Fatma **ARSLAN**, Gazi University

Prof. Vedat **TÜRKOĞLU**, Van Yüzüncüyıl University

Prof. Ayhan **ORAL**, Çanakkale Onsekiz Mart University

Prof. Azra **BOZCAARMUTLU**, Bolu İzzet Baysal University

Prof. Mehmet **DOĞRU**, Dicle University

Prof. T. Abdulkadir **ÇOBAN**, Erzincan University

Assoc. Prof. Melike **YILDIRIM AKATIN**, Karadeniz Technical University

Assoc. Prof. Harun **BUDAK**, Atatürk University

Assoc. Prof. Nalan **ÖZDEMİR**, Erciyes University

Assoc. Prof. Gülşah **ŞANLI MOHAMED**, İzmir Institute of Technology

Assoc. Prof. Yakup **KOLCUOĞLU**, Karadeniz Technical University

Assoc. Prof. Seyhan **ULUSOY**, Süleyman Demirel University

Assoc. Prof. Nilay **BERELİ**, Hacettepe University

Assoc. Prof. Barış **BİNAY**, Gebze Technical University

Assoc. Prof. Lokman **UZUN**, Hacettepe University

Assoc. Prof. Aytuğ **OKUMUŞ**, Ankara University

Assoc. Prof. Emirhan **NEMUTLU**, Hacettepe University

Assist. Prof. Şebnem **EŞSİZ**, Kadir Has University

Assist. Prof. Barbaros **DİNÇER**, Recep Tayyip Erdoğan University

Dr. Mehtap **YAKUT ÖZGÜR,** Ankara University

Dr. Gökhan **GÜNGÖR**, Microbiota

Murat Can **ANARAT**, Consultancy Firm

Scientific Committee

Prof. Jean-François **GERARD,** National Institute of Applied Sciences of Lyon

Prof. Alvaro **MATA,** Queen Mary University of London

Prof. Ahmed **JASHARI,** University of Tetova

Prof. Hatem **MAJDOUB,** University of Monastir

Prof. Alain **THIERRY**, Institute of Cancer Research of Montpellier

Prof. Miral **DİZDAROĞLU**, NIST

Prof. Ayşe **OGAN**, Marmara University

Prof. İlhami **GÜLÇİN**, Atatürk University

Prof. Uğur **SEZERMAN**, Acıbadem University

Prof. Leman **TARHAN**, Dokuz Eylül University

Prof. Şükrü **BEYDEMİR**, Anadolu University

Prof. İsmail **KIRAN**, Eskişehir Osman Gazi University

Prof. Refiye **YANARDAĞ**, İstanbul University

Prof. Hülya **YAĞAR**, Trakya University

Prof. Nagihan **SAĞLAM ERTUNGA**, Karadeniz Teknik University

Prof. Figen **ZİHNİOĞLU**, Ege University

Prof. Barbaros **NALBANTOĞLU**, Yıldız Teknik University

Prof. İsmail **ÖZMEN,** Süleyman Demirel University

Prof. Adil **DENİZLİ,** Hacettepe University

Prof. Özlem **YILDIRIM,** Ankara University

Prof. Handan **YAVUZ**, Hacettepe University

Prof. Nilgün **ANCIN**, Ankara University

Prof. Gül **ÖZTAŞ,** Ankara University

Prof. Turgay **SEÇKİN,** İnönü Üniveristesi

Prof. Aydan **YILMAZ**, Selçuk University

Prof. Burhan **ATEŞ**, İnönü University

Assoc. Prof. Andreas G. **TZAKOS,** University of Ioannina

Assoc. Prof. Fatma **EROĞLU**, Ankara University
Assoc. Prof. Melike **KALKAN,** Ankara University
Assoc. Prof. Gökhan **DEMİREL,** Gazi University
Assoc. Prof. Özlem **BİNGÖL ÖZAKPINAR**, Marmara University
Assoc. Prof. Gözde **AYDOĞDU TIĞ**, Ankara University
Assist. Prof. Georg A. **FEICHTINGER**, Leeds University

Local Organizing Committee

Prof. Kaan C. **EMREGÜL,** Ankara University (Chairman)
Prof. Şenay **ŞANLIER**, Ege University
Assoc. Prof. Sedat **ODABAŞ,** Ankara University (Vice Chair)
Assoc. Prof. Serdar **KARAKURT,** Selçuk University
Assist. Prof. Burak **DERKUŞ**, Eskişehir Osmangazi University
Assist. Prof. Çağlar **ELBÜKEN**, Bilkent University
Assist. Prof. Yavuz Emre **ARSLAN**, Çanakkale Onsekiz Mart University
Nurettin Mete **KALELİ**
Mesutcan **ŞAHİN**
Hüseyin **KORKMAZ**
Sercan **CANTÜRK**
Mehmet Sait **YILMAZ**
Can Berk **CAMCI**
İsmail **KUTLU**
Sıla **BİLGİN**
Ece **KARAKAYA**
Dilara **KURT**
Açelya **ÖZBOŞNAK**
Melis **IŞIK**

Supramolecular Engineering Of Functional Biomaterials For Advanced Tissue Engineering

Alvaro MATA

There is great interest to develop new materials with properties that resemble those of biological systems such as hierarchical organization, the capacity to grow or self-heal, and the ability to guide complex biological processes. To this end, supramolecular chemistry offers an exciting opportunity to grow such materials from the bottom-up using molecules and processes found in nature. However, the ability to transform molecular and nano-scale design into functional devices with practical utility at the macroscale remains a challenge. The talk will describe new strategies that integrate supramolecular chemistry with engineering principles to develop practical materials capable of acquiring hierarchical organization, being bioactive, growing, and exhibiting tuneable mechanical properties. These materials are being used towards new regenerative therapies of tissues such as enamel, bone, and blood vessels as well as more biologically relevant *in vitro* models for applications such as cancer and neurological disorders.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

POLYMER FIBERS-BASED ELECTROSPUN SCAFFOLDS FOR NEURAL TISSUE ENGINEERING

*Jean-François GERARD**

Co-authors: L. CHAVEZ LINS[*], A. HIDALGO IDALBA ANDREINA[*], F. WIANNY[**], C. DEHAY[**], J. DUCHET[*]
[(*)] Ingénierie des Matériaux Polymères - UMR 5223 CNRS
Université de Lyon - INSA Lyon / F69621- Villeurbanne (France)
[(**)] Stem Cell & Brain Research Institute - U1208 INSERM
Université de Lyon - Université Lyon 1 / F69500 – Bron (France)

Electrospinning process is one of the most promising routes for the design and development of smart textiles based on polymer nanofibers. From a proper selection of the electrospining process parameters and polymers, (multi)functional textiles could be proposed. In this lecture, we will show how biobased polymers, such as PLA-based, and biodegradable polymers, such as PBAT, can be used to prepare electrospun scaffolds. In the first part, electrospinning is applied to neat polylactic acid (PLA) and to PLA-based blends, i.e. PLA/polyethylene glycol-b-polylactic acid block copolymers and PLA/PEG homopolymer. Electrospun membranes exhibit fibers having diameters from 110 to 310 nm depending on the composition and large amounts of porosity (about 80% vol.) which are required for cell culture application. In vitro degradation, as well as the hydrophilicity of the electrospun scaffolds, can be finely tuned from material composition. Fluorescence microscopy shows that the PLA electrospun fibers based scaffolds are good candidates for the survival and proliferation of neural stem cells. Even if the introduction of hydrophilic segments, i.e. polyethylene glycol from PLA-b-PEG block copolymer, leads to the the same level of proliferation than PLA-based membranes, the PLA/PLA-b-PEG electrospun membranes exhibited the suitable hydrolytic degradation required for implantable scaffolds. The second part deals with the development of biodegradable PBAT electrospun membranes with potential applications in the field of smart textiles. As mentioned previously, the fiber morphology is strongly dependent on the tip-collector distance, concentration, and applied voltage. Smooth fibers and beads free membranes could be prepared and analyzed to establish morphology-properties relationships. PBAT membranes having the best thermal and mechanical properties were selected as host of a curli protein which is able to complex heavy metals. In fact, by electrospinning, porous membranes exhibiting a large surface-to-volume ratio could be proposed for chelation of pollutants such as nickel.

Related papers:

[1] 'Influence of hydrophilic-hydrophobic bioabsorbable electrospun scaffolds on neural stem cell culture'. L.C. Lins, F. Wianny, S. Livi, C. Dehay, J. Duchet, J.F. Gérard. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 105 (8), 2376-2393 (2017)

[2] 'Development of bioresorbable hydrophilic-hydrophobic electrospun scaffolds for neural tissue engineering'. L.C. Lins, F. Wianny, S. Livi, A. Hidalgo, I. Dalba Andreina, C. Dehay, J. Duchet, J.F. Gérard. Biomacromolecules, 17 (10), 3172–3187 (2016)

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Discovery of a Cancer Biomarker, Repurposing a Drug and Development of Smart Prodrugs Against Cancer

Andreas G. Tzakos[a] and Serdar KARAKURT[b]

University of Ioannina, Department of Chemistry, Ioannina, GREECE

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY

agtzakos@gmail.com

Despite emergence of new systemic therapies, metastatic melanoma remains a challenging and often fatal form of skin cancer. The renin-angiotensin system (RAS) is a major physiological regulatory pathway controlling salt-water equilibrium, intravascular volume and blood pressure. We will present that the RAS has both oncogenic and tumour suppressor functions in melanoma. Furthermore, we will indicate that pharmacological inhibition of AT2R may provide therapeutic opportunities in melanomas expressing this receptor and AGTR1 CpG island methylation in serum may serve as a novel biomarker of metastatic melanoma. Furthermore, we will highlight several prodrugs and formulates that we have developed as potent anticancer agents.

2EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Yeast as Oxidation-Reduction Cell Factories: From Whole Cells to Stereospecific Enzymes

Anju Chadha*[1,2], Vinay Kumar K[1], Uma Mahesh M.V.N[1], Jerrina J.P[1], Leena K[1], Archana S[1], Debayan C[1], Joshua C[1]

[1]*Bioorganic Chemistry laboratory, Department of Biotechnology and* [2]*National Center for Catalysis Research, IITM, Chennai 600036, India*

anjuc@iitm.ac.in

Biocatalysed reactions, mediated by enzymes which are chemo-, regio- and stereo-specific and function under mild conditions, require reduced quantites of organic solvents thus providing a very valuable route for green chemistry. In fact, other media are being studied to make biocatalysed reactions greener and more efficient[7]. The yeast, *Candida parpasilosis* ATCC 7330, a rich source of oxido-reductase enymes is an established versatile biocatalyst from our lab[1]. It has been shown to reduce a variety of carbonyl compounds to yield optically pure synthons[2-5]. The yeast also reduces imines to optically pure amines[6]. Oxidation of some alcohols has also been demonstrated by the whole cells of this yeast[8,9].

While whole cells have the advantage of containing cofactors making them valuable biocatalysts for synthesis, pure enzymes are essential to understand their structure and mechanism in order to engineer them for more efficient catalytic performance. Our efforts towards this have resulted in the purification of the following reductases: recombinant (*R*) [CpCR][10,11] and (*S*)-specific [SRED][12,13], and a wild type (*S*)-specific reductase[14] from *Candida parapsilosis* ATCC 7330. SRED asymmetrically reduces ketones with excellent enantiospecificity (ee > 99%) while α-ketoesters show moderate enantiospecificity (ee 70%) in the presence of NADPH and there is a minimal reduction of aldehydes. CpCR, on the other hand reduces aryl α–ketoesters to their respective (*R*) alcohols but preferentially reduces aliphatic and aryl aldehydes to primary alcohols. The reduction of α-ketoesters with SRED can occur with either NADPH or NADH, but for ketone reduction SRED requires NADPH specifically. The reduction of aldehydes by CpCR is cofactor (NADPH) specific unlike the α–ketoesters which show dual specificity. CpCR is strictly metal dependant (Zn^{++}) for its activity whereas the SRED is not. Taken together, SRED and CpCR offer substrates which on asymmetric reduction give products of opposite absolute configurations. *In silico* studies show that in SRED, the *Pro S* hydride attacks the carbonyl carbon of the substrate whereas in CpCR there is *Pro R* hydride transfer. The importance of both the above enzymes in xenobiotic metabolism and thereby effect on some drugs is being studied[15].

The wild type (*S*)-reductase responds to heat treatment better than it does to ammonium sulfate precipitation for purification and is NADH specific. It prefers ketones substrates as compared to aldehydes[16]. The imine reductase, CpIM1[17] from this *Candida* belongs to Mu-crystallin/Ornithine cyclodeaminase family. It catalyzes alkylamination of ketoacids and ketoesters.

References:

1) Chadha A, Venkataraman S, Preetha R, Padhi SK. *Bioorg Chem.* **2016**, 68, 187–213.
2) Baskar B, Pandian N.G, Priya K, Chadha A. *Tetrahedron: Asymmetry* **2004,** 15, 3961-3966.
3) Kaliaperumal T, Kumar S, Gummadi S.N, Chadha A. *J. Ind. Microbial Biotechnol.* **2010,** 37, 159-165.
4) Stella S, Chadha A. *Catal. Today*. **2012**, 198(1), 345-352.
5) Venkataraman S, Roy R.K, Chadha A. *Appl Biochem Biotechnol.* **2013**, 171(3), 756-770.
6) Vaijayanthi T, Chadha A. *Tetrahedron Asymmetry*. **2008**, 19(1), 93-96.
7) Archana S, unpublished results, IITM [**2019**].
8) Sivakumari T, Chadha A. *RSC Adv.* **2014**, 4(105), 60526-60533.
9) Sivakumari T, Preetha R, Chadha A. *RSC Adv.* **2014**, 4(5), 2257-2262.
10) Aggarwal N, Mandal PK, Gautham N, Chadha A. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* **2013**, 69(3), 313–5.
11) Aggarwal N, Ananthathamula R, Karanam VK, Doble M, Chadha A. *Mol Catal.* **2018**, 460, 40–5.
12) Sudhakara S, Chadha A. *Org Biomol Chem.* **2017**, 15(19), 4165–71.
13) Joshua C, BTech/MTech project [**2019**].
14) Debayan C, BTech/MTech project [**2019**].
15) Leena K, unpublished results, IITM [**2019**].
16) Jerrina J. P, unpublished results, IITM [**2019**].
17) Uma Mahesh M.V.N, unpublished results, IITM [**2019**].

2.EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Gene-activated matrices (GAMs)- Concept, Limitations And Future Developments

Georg Feichtinger

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Surface-enhanced Raman spectroscopy (SERS): An adventure from plasmonic metals to organic semiconductors as SERS platforms

Gökhan Demirel

Bio-inspired Materials Research Laboratory (BIMREL), Department of Chemistry, Gazi University, 06500 Ankara, Turkey

Email: gdemirel@gazi.edu.tr

The analysis of ultralow concentrations of (bio-)chemical molecules is relevant in several fields including medicine, environmental science and homeland security.[1] Traditional approaches to molecular detection rely on optical, electrochemical, electronic and gravimetric methodologies.[2] Among these platforms, Surface-enhanced Raman Spectroscopy (SERS) is an ideal surface-sensitive technique allowing non-destructive molecular analysis with high sensitivity and selectivity approaching single-to-few molecule detection.[3] Unfortunately, the applicability of SERS is rather limited, which is mainly due to the lack of highly sensitive SERS platforms with good stability and reproducibility. In line with this, metal nanoparticles (e.g., Au, Ag, and Cu) have been extensively exploited as SERS active platforms. Although the utilization of metallic nanoparticles in SERS is simple and cost-effective, the poor controllability of the structures and limited formation of hot spots in the detection zone leads to discrepancy in the resulting SERS signals. For these reasons, in the past few years, researchers have focused on fabricating 3-dimensional (3D) SERS platforms, which increase the adsorption of analyte molecules and facilitate hot spot formation in all three dimensions. However, the fabrication of 3D SERS platforms is mostly expensive and technologically demanding. Therefore, the discovery of non-metal alternative approaches is of great interest not only to widen SERS applications but to further elucidate fundamental questions.[4,5] In this presentation, I will describe our processes for creating effective SERS platforms (plasmonic and non-plasmonic) and present results concerning their applications.

References:

Hudson, S.D.; Chumanov, G. *Anal. Bioanal. Chem*. **2009**, *394*, 679.
Chen, A.; Chatterjee, S. *Chem. Soc. Rev*. **2013**, *42*, 5425.
Demirel, G.; Usta, H.; Yilmaz, M.; Celik, M.; Alidagi, H.A.; Buyukserin, F. *J. Mater. Chem. C* **2018**, *6*, 5314.
Yilmaz, M.; Ozdemir, M.; Erdogan, H.; Tamer, U.; Sen, U.; Facchetti, A.; Usta, H.; Demirel, G. *Adv. Funct. Mater*. **2015**, *25*, 5669.
Yilmaz, M.; Babur, E.; Ozdemir, M.; Gieseking, R.L.; Dede, Y.; Tamer, U.; Schatz, G.C.; Facchetti, A.; Usta, H.; Demirel, G. *Nat. Mater*. **2017**, *16*, 918.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The impact of exosomes in brain disorders: from pathogenesis to clinical application

Şermin GENÇ[1, 2]

[1] *İzmir Biomedicine and Genome Center*
[2] *Dokuz Eylül University*

Exosomes are small membrane vesicles released under normal or pathological conditions from various cells. They are distinguished from membrane vesicles by their size (40-100 nm). Features of exosomes like cytoplasm surrounded with lipid bilayer. Their release occurs by fusion of multi-vesicular structures and differentiation from the plasma membrane. Today, exosomes have been shown to be present in all body fluids, including brain-spinal fluid. They carry several cargo molecules including mRNA, protein and miRNA. In fact, RNA-containing exosomes were described in the late 1900's. These RNA molecules are transferred to other cells and the transferred mRNA molecules in the target cell are converted to protein while the miRNAs modulate gene expression.

In recent years, several studies showed that exosomes have important role in the development and progression of brain disorders. Additionally, exosomal cargo molecules such as miRNAs can be isolated from blood and used as potential novel diagnostic and prognostic biomarkers for several brain diseases. Also, exosomes are good drug-carrier due to their low immunogenicity and toxicity, high stability in circulation and tissue, and accumulation in the target tissue.

References

1. Zhang et al(2019). Nat Rev Neurol 10.1038/s41582-018-0126-4.
2. Shi et al(2019). Nat Rev Neurol Prog Neurobiol 10.1016/j.pneurobio.
3. Osorio-Querejeta et al(2018).Front Mol Neurosci 10.3389/fnmol.
4. Osier et al(2018). Mol Neurobiol. 10.1007/s12035-018-1054-4.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Microfluidic Sorting Of Sperm For Applications In Assisted Reproductive Technologies

Utkan DEMIRCI

Micro- and nano-scale technologies can have a significant impact on medicine and biology in the areas of cell manipulation, diagnostics and monitoring. At the convergence of these new technologies and biology, we research for enabling solutions to real-world problems at the clinic. Emerging nano-scale and microfluidic technologies integrated with biology offer innovative possibilities for creating intelligent, mobile medical lab-chip devices that could transform diagnostics and monitoring, tissue engineering and regenerative medicine. Male infertility is a reproductive disease, and existing clinical solutions for this condition often involve long and cumbersome sperm sorting methods, including preprocessing and centrifugation-based steps. These methods also fall short when sorting for sperm free of reactive oxygen species, DNA damage, and epigenetic aberrations. Existing platforms suffer from structural complexities, i.e., pumps or chemoattractants, setting insurmountable barriers to clinical adoption. Inspired by the natural filter-like capabilities of the female reproductive tract for sperm selection, a model-driven design—featuring pillar arrays that efficiently and noninvasively isolate highly-motile and morphologically normal sperm, with lower epigenetic global methylation, from raw semen—is presented. The microfluidic sperm sorters that we created, such as the Simple Periodic ARray for Trapping And isolatioN (SPARTAN), modulate the directional persistence of sperm, increasing the spatial separation between progressive and non-progressive motile sperm populations. They lead to results within an unprecedentedly short 10-minute assay time. With over 99% motility of sorted sperm, a 5-fold improvement in morphology, 3-fold increase in nuclear maturity, and 2–4-fold enhancement in DNA integrity, SPARTAN offers to standardize sperm selection while eliminating operator-to-operator variations, centrifugation, and flow. Some of these innovative microfluidic devices have been translated into FDA approved and CE-marked products, where they have been widely used by fertility clinics around the world to serve patients, leading to an estimated 10,000+ live births globally.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Integrative Network Modeling in Human Diseases

Nurcan Tuncbag

Orta Dogu Teknik Universitesi, Enformatik Enstitusu, Cankaya 06800 Ankara Turkey

ntuncbag@metu.edu.tr

With the accumulation of different types of patient-specific big data in biology and medicine, and parallel developments in technology, we are in an age of the systems science rather than the naïve reductionism. Making sense of these data is a daunting task which created opportunities for computational scientists to step in and make big contributions in biology and medicine. Beyond the list of molecules from each data type, there is a necessity to consider multiple data jointly and to reverse engineer the relationships between these molecules with personalized network-based approaches. In my talk, I will introduce the Omics Integrator software that solve the prize-collecting Steiner forest problem to integrate a variety of 'omic' data as input and identify putative underlying molecular pathways. Next, we use Omics Integrator to address a critical question for tumor specific therapy: can network-based approaches reveal patient specific pathways and targets? A proof of principle of this strategy will be presented on a group of Glioblastoma multiforme patients (GBM, the most common and aggressive type of malignant human brain tumor).

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Biotechnological Production of Terpenoid Indole Alkaloids by *Catharanthus roseus* Hairy Roots

Roukia Benyammi[1,2*], Majda Salaoui-Khelifi[1], Abdelkader Morsli[1], Stéphane Desobry[3], Lakhdar Khelifi[1].

LRGB, ENSA (ES1603), Algiers, Algeria.
ENS – Kouba, Algiers, Algeria.
ENSAIA of Nancy, France.

*Corresponding author email: benyammi.r.s@gmail.com

Abstract

Catharanthus roseus (L.) G. Don (Apocynaceae) is still one of the most important sources of terpenoid indole alkaloids including anticancer molecules as vincristine and vinblastine and antihypertensive molecule ajmalicine. The main limiting hurdle to produce sufficient amount of these compound are the low yield. Catharanthine and ajmalicine are the important precursors of final compounds and their increase can lead to enhance levels of molecules of targets and interest. Hairy roots are an excellent system to study the regulation mechanisms of these precursors. In this work, we report the induction and the establishment of different hairy root lines in *C. roseus* by using *A. rhizogenes*. Subsequently, the growth kinetics and accumulation of two monoterpenoid indole alkaloids (ajmalicine and catharanthine) of the three selected hairy root lines (LP10, LP21 and L54) were measured throughout a 35-day culture cycle. The methanolic extract for each line in different times during culture cycle is analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Maximum accumulation of the alkaloids is recorded for LP10 line in which the peak of ajmalicine and catharanthine accumulation reached to 3.8 and 4.3 mg/g dry weight (DW), respectively. This increase coincides with an exponential growth phase. Our results, suggest that the evolution of accumulation of ajmalicine and catharanthine are positively correlated with the development of the biomass growth. Significantly for LP10 line the most promising line to continue optimizing the production of TIAs. Additionally, the end of exponential phase remains the best period to apply the elicitors' treatment in the following steps of our research.

.

Keywords: Agrobacterium rhizogenes, medicinal plant, terpenoid indole alkaloids, growth kinetics, secondary metabolites, LC-MS/MS.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Chemical Analysis and Antioxidant Effect of Phenolic-Rich Extracts From Three Endemic Medicinal plants

Krimat Soumeya[a], Kesouri Aicha[a], Nouasri Ahmed[a], Metidji Hafidha[a], Dahmane Dahmane[a]

[a]*Laboratory of bioactive products and biomass valorization research. Ecole Normale Supérieure- kouba, Algiers, Algeria*

krimatsoumia.2012@gmail.com

This work concerns the study of phytochemical and antioxidant properties of three endemic species belonging to the Lamiaceae family growing in Algeria: *Salvia chudaei* Batt. et Trab., *Lavandula antineae* Maire and *Origanum glandulosum* Desf. The aim of the phytochemical analysis was the characterization of different classes of secondary metabolites, the quantification of total phenolic and total flavonoid contents and the chemical analysis of phenolic compounds. The antioxidant activity was carried out using three methods: DPPH radical scavenging, β-carotene bleaching and reducing power. The phytochemical screening of the extracts of aerial parts of three plants reavealed a remarkable presence of flavonoids, tanins, sterols, terpens, saponins, anthraquinons with total absence of alkaloids. The colorimetric assay showed that the contents of total phenolic and flavonoid of hydromethanolic extracts and their fractions differ from one plant to another and that diethyl ether and ethyl acetate fractions showed the highest polyphenols content which ranged between 537.58±16.92 and 54.49±6.76 mg EAG/g ES. The chromatographic characterization of hydromethanolic extracts by HPLC-DAD revealed the presence of phenolic acids, flavanols, flavanons, flavonols and flavons. The results of antioxidant power determined by three tests clearly revealed that the extracts of three plants, particularly the fractions obtained by diethyl ether and ethyl acetate, are good hydrogen and electron donors, capable of scavenging the free radicals (DPPH •) and peroxyl radicals (LOO •) resulting from oxidation of linoleic acid. A good correlation was also found between phenolic content in plants and their antioxidant capacity.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Antiviral Effect of Uzungol Propolis Ethanol Extract Against Herpes Simplex Virus Type 1

Merve CORA[a], Neşe KAKLIKKAYA[a], Sevgi KOLAYLI[b], Celal Kurtuluş BURUK[a], Serbülent ÜNSAL[c]

[a]*Karadeniz Technical University, Faculty of Medicine, Department of Medical Microbiology, Turkey*

[b]*Karadeniz Technical University, Faculty of Science, Department of Biochemistry, Turkey*

[c]*Karadeniz Technical University, Facult of Medicine, Department of Biostatistics and Medical Informatics, Turkey*

merve.tokdemir@yahoo.com

Aim: Propolis is a resinous substance in which the materials that the bees gather from the plants mix with the enzymes in their own saliva. Propolis is used by bees for variety of purposes including as construction material, for protection, and the balance of heat and moisture of the hive. It has also been used in traditional medicine for centuries because of its pharmacological properties. Both the research studies on propolis and its use in the treatment of various diseases continuously. The aim of this study was to evaluate the antiviral effect of propolis sample obtained from Uzungol (Trabzon, Turkey)on HSV-1.

Method: Propolis extract sample was prepared with 70% ethanol. The ethanol was removed by evaporation, and the water was removed by lyophilization from the extract, and then the dry matter was dissolved by using DMSO. The total phenolic content in the extract was determined, and the phenolic components were analyzed by HPLC-UV. The cytotoxic effect of the extract on VERO cells was determined by trypan blue staining and MTT method. The non-cytotoxic concentration of the extract to VERO cells was used in antiviral activity assays. The antiviral effect of the extract on HSV-1 was investigated by MTT, RT-PCR and plaque reduction methods. The significance of the data obtained was analyzed statistically.

Results: The total phenolic content of Uzungol propolis ethanol extract was found to be 166.908 mg gallic acid equivalent / g propolis. HPLC-UV analysis revealed that the most common phenolic component in the extract was chrysin (68.12 mg extract / g sample) followed by caffeic acid phenyl ester (46.02 mg extract / g sample). The extract was not cytotoxic to VERO cells at concentrations of 100 µg/mL and below. The antiviral effects of the extract on HSV-1 at 50 µg/mL in MTT assay, and at 100 µg/mL concentration level in RT-PCR and plaque reduction assays were found statistically significant ($p<0.05$).

Conclusion: Although drugs used in infections caused by HSV-1 are effective in primary infection, they may be insufficient in recurrent infection. Therefore, antiviral and vaccine development studies continue for treatment of HSV-1 infections. In this study, it has been shown that Uzungol propolis can be considered as a candidate in drug development studies for the treatment of HSV-1 infections.

This study was supported by Karadeniz Technical University Scientific Research Projects Unit (Project #: TDK-2016-5602).

Key Words: Antiviral, HPLC, HSV-1, MTT, Propolis, RT-PZR

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Comparison of the Potential of Inducing Apoptosis of Essential Oils Obtained from the Natural and Cultured form of *Origanum acutidens* in Lung Cancer Cells

Ayşe Erdoğan[a], Aysun Özkan[b]

[a]*Alanya Alaaddin Keykubat University, Faculty of Engineering, Genetic and Bioengineering Department, Alanya, Antalya, Turkey*
[b]*Akdeniz University, Science Faculty, Biology Department, Antalya, Turkey*

ayse.erdogan@alanya.edu.tr

According to the World Health Organization (WHO) report, lung cancer is the first in men and third in women in cancer-related deaths.[1] Chemotherapy, which is one of the most frequently used methods in the treatment of lung cancer, cannot achieve the desired success. The failure of existing anti-cancer drugs to treat lung cancer reveals the need for the development of new chemotherapeutics.
The origin of many drugs with known antineoplastic properties is plants. Today, research on the anticancer effects of natural compounds is of great interest. It is known that apoptosis is one of the most important mechanisms used in cancer treatment. Bax, a pro-apoptotic protein, and anti-apoptotic Bcl-2 balance are known to be important in determining whether cells survive or die.
The aim of this study is to reveal apoptotic effect of essential oils obtained from natural and cultured form of *Origanum acutidens* (*O. acutidens*), which is an endemic species grown in Turkey, in lung cancer cells, H1299.
Caspase-3/7 activity were determined using the fluorometric ApoTox-Glo^{TM} Triplex Assay kit (Promega). mRNA was isolated using 'RNeasy Mini Kit (Qiagen)' from the cells that were treated with essential oils for 24 h. mRNA isolated from the cells was converted to cDNA under appropriate conditions using 'Titan One Tube RT-PCR System Kit'. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) products were analyzed by 2.5% gel electrophoresis. Adobe Photoshop CS4 programme was used for band density analyzing.
When the essential oils from natural and cultured forms of *O. acutidens* were exposed to H1299 cells, caspase 3/7 activities increased 3 and 2 fold, respectively. When the essential oil obtained from cultured form of *O. acutidens* was applied to H1299, it was found that Bcl-2 mRNA expression decreased by 57.6%, while Bcl-2 expression was reduced by 13.6% when the essential oil obtained from natural form was applied. In addition, the application of the essential oil of both forms increased Bax mRNA expression in H1299. However, it was determined that cultured form treatment increased Bax expression more than natural form treatment. Thus, it has been shown that treatments of essential oils obtained from both natural and cultured form induced apoptosis by activating caspase 3/7 and increasing Bax/Bcl-2 ratio in H1299.

References:

www.wcrf.org.2012

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Production, Characterization of Recombinant Laccase from *Bacillus licheniformis* O12 and Effects of Some Organic Solvents and Surfactants on Enzyme Activity

Arzu ÖZTÜRK KESEBİR[a], Deryanur KILIÇ[a], Ö.İrfan KÜFREVİOĞLU[a], Melda ŞİŞECİOĞLU[b], Ahmet ADIGÜZEL[b]

[a]Atatürk University, Faculty of Science, Department of Chemistry
[b]Atatürk University, Faculty of Science, Department of Molecular Biology and Genetics
25240-Erzurum/TURKEY
okufrevi@atauni.edu.tr

Multiple copper-containing laccase enzymes (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) can oxidize both phenolic and non-phenolic lignin-related compounds, as well as environmental pollutants that are resistant to biodegradation. They use molecular oxygen as an electron acceptor and do not require toxic peroxide hydrogen, so laccase enzymes have recently been a very interesting area in research.[1] Since laccase enzymes are characterized by low substrate specificity, they can also oxidize environmental pollutants which have resistance to biological degradation.[2] This oxidation ability of laccase enzymes increases the demand for these enzymes in some industrial and biotechnological processes.

In this study, the laccase enzyme gene was amplified by PCR method using protein gene specific primers with DNA obtained from *Bacillus licheniformis* 012 local strain. The laccase gene was transferred to the pET SUMO vector by TA cloning. The obtained recombinant DNA was transformed into competent OneShot Mach1 E. coli cells by heat-shock method. Positive colonies were determined by colony PCR and these plasmids were isolated. Sequence analyses of the recombinant plasmid were performed with vector primers. These plasmids were expressed in *E. coli* BL21DE3 cells. Recombinant fusion laccase enzyme was purified by Ni-NTA affinity column. Optimum pH, optimum temperature and optimum ionic strength values of the purified enzyme were determined. Also, the effects of some organic solvents and surfactants on the enzyme were investigated.

As a result, recombinant laccase enzyme was successfully expressed in soluble form in 1mM IPTG and at 37°C by using pET SUMO vector. Optimum ionic strength, optimum temperature and optimum pH values of the sumo-fusion enzyme were determined as 0.1 M, 92°C and pH 5.0 respectively. It has been observed that effects of organic solvents on enzyme activity are more than influence of surface active agents but recombinant laccase enzyme activity is not completely lost.

REFERENCES

Mayer AM. Polyphenol oxidases in plants-recent progress. Phytochemistry, 1987; 26: 11–20.

Paloheimo M, Valtakari L, Puranen T, Kruus K, Kallio J, Mantyla A, et al. Novel laccase enzyme and use thereof. USPTO Applicaton:20060063246, Class: 435183000 (USPTO), 2004.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Detection of aflatoxins, comparison of determination methods and current approaches

Hatice PALUZAR

Trakya University, Arda Vocational School, Department of Chemistry and Chemical process technology, Edirne/ TURKEY

haticepaluzar@trakya.edu.tr

Microfungi, known as mold, produce toxic substances called mycotoxins in dairy products, cereals, animal feeds and other dry foods. The most common of these toxic substances in foods are aflatoxins. Aflatoxins are toxic metabolites produced mainly by *Aspergillus flavus* and *Aspergillus paraciticus* and 18 different types of aflatoxins have been identified [1]. The most common of these are aflatoxins known as B1, B2, G1 and G2. In addition, aflatoxin M1 and M2 are afalatoxins in milk. The various food products contaminated with aflatoxins include cereals, milk, nuts, figs, cheese, cotton, almonds, spices, corn, red pepper and animal feed [2]. Aflatoxins were detected in animal products such as milk, milk and eggs obtained from animals consumed aflatoxin-contaminated feed. Therefore, people consuming these animal products and other aflatoxin-containing foods are exposed to aflatoxins. People who are exposed to these toxins frequently and continuously have many serious health problems, especially liver cancer [3].

Such toxins should not exceed certain limits in food. In many countries, including Turkey, has the legal limits for aflatoxin values in food. In our country, aflatoxin analysis is carried out in Food Analysis Laboratories by using high cost kits obtained from abroad for preconcentrated and then analyzed by High Pressure Liquid Chromatography (HPLC), Fluorimetry, ELISA (EnzymeLinkedImmunoSorbentAssay) and RIA (RadioImmunoAssay) methods. Unfortunately, many countries around the world that are affected by the aflatoxin problem do not have ready access to these expensive equipment and require alternate, readily available and simple detection methods that may be used by small holdings farmers in developing countries, it is important to develop alternative analysis methods in order to create cheap, sensitive and simple analysis methods and make on-site and easy analysis by small-scale farmers. In this study, the advantages and disadvantages of the present analysis methods and the new analysis methods for aflatoxins (biosensors, immunoaffinity columns, ….) are presented and current approaches for aflatoxin analysis have been examined.

References:

Ehrlich, K.C., Montalbano, B.G., Cotty, P.J. *Fung. Gen. and Bio*. **2003**, *38*, 63.
Shephard, G.S. *Analytical and Bioan. Chem*. **2009**, *395*, 1215.
Gong, Y.Y., Watson, S., Routledge, M.N. *Food Safety*, **2016**, **4**, 14.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Methylglyoxal induces Glyoxalase 1 expression in high glucose but not in low glucose media in vascular smooth muscle cells

Mustafa Kırça

Kütahya Health Sciences University, Department of Medical Biochemistry, Kütahya, Turkey

mustafa.kirca@ksbu.edu.tr

Objectives: Diabetes is a global health problem and methylglyoxal (MGO) is a reactive, AGE precursor dicarbonyl molecule, found elevated in diabetic patients[1]. MGO is a glycolysis by-product and raised MGO concentration results in enhanced protein and DNA damage, contributing to diabetic complications such as cardiovascular diseases[2]. MGO is detoxified by the glyoxalase system and the main enzyme of this system is Glyoxalase 1 (Glo1). In this study, the effect of MGO, the substrate of Glo1, on Glo1 protein expression were explored under low or high glucose media in vascular smooth muscle cells (VSMCs), those play a prominent role in vascular diseases. Telmisartan and irbesartan were suggested to be protective against MGO, hence their effects were also studied.

Materials and Methods: Primary cultured VSMCs were isolated from rat aorta. MGO-treated cells (200 µM) were incubated in low (5,5 mM) or high glucose (25 mM) media for 48 hours with or without telmisartan or irbesartan (both 10 µM). Glo1 protein expression was measured by ELISA technique as triplicates.

Results: Our results showed that MGO did not affect Glo1 expression in low glucose media. High glucose alone significantly alleviated Glo1 expression. Surprisingly, MGO treatment in high glucose media increased Glo1 expression nearly %96. Telmisartan and irbesartan were not found effective to restrain MGO action in terms of Glo1 protein expression.

Conclusions: Diminished Glo1 expression upon exposure to high glucose is in parallel to the findings observed in diabetic patients. However, the reason behind that while MGO is not effective on Glo1 expression under low glucose but high glucose might be because cells defend themselves to MGO-induced toxicity and death. In some respects, VSMCs have similarities with cancer cells like enhanced proliferation in high glucose conditions which are toxic to normal cells[3-4]. Thus, MGO-induced elevation of Glo1 expression in high glucose media in VSMCs might be attributable to the behaviour of cancer cells as Glo1 overexpression has been determined in many cancer types to avoid MGO-induced cell death.

Key Words: methylglyoxal, Glo1, telmisartan, irbesartan, high glucose.

References:

Hanssen, N. M. J.; Scheijen, J.; Jorsal, A.; Parving, H. H.; Tarnow, L.; Rossing, P.; Schalkwijk, C. G. *Diabetes*. **2017**, *66(8),* 2278.

Rabbani, N.; Thornalley, P. J. *Biochem Soc Trans.* **2014**, *42(2)*, 425.

Marsboom, G.; Archer, S. L. *Circ Res.* **2008**, *103(10)*, 1047.

Duan, W.; Shen, X.; Lei, J.; Xu, Q.; Yu, Y.; Li, R.; Ma, Q. *BioMed Res Int.* **2014**, 461917.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

PREPARATİON OF PEMETREXED LOADED ALBUMIN NANOPARTİCLES AND INVESTIGATION OF THEIR EFFICACY IN LUNG CANCER CELL LINES

Ayşe Ünal,[a] Şenay Hamarat Şanlıer[a,b]

[1]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[2] *Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

ayseunal.93.35@gmail.com

Abstract

Albumin is the most abundant spherical protein in plasma and contains almost all amino acids in its structure. Furthermore, albumin is synthesized in the liver at a rate of about 0.7 mg / h. Albumin as drug delivery system; It is preferred in nanoparticle structure due to its protein structure, biodegradability, nontoxic, biocompatible and easily available. Pemetrexed is one of the cytotoxic agents used for non-small cell lung cancer and is a potent inhibitor of thymidylate synthase. However, life-threatening complications may occur after Pemetrexed administration. In this study, it was aimed to target the lung cancer site with magnetic nanoparticles. Therefore, pemetrexed loaded magnetic albumin nanoparticles were prepared.

Albumin nanoparticles were synthesized by desolvation method using bovine serum albumin. During the preparation of the nanoparticle, genipin was used as cross-linker instead of toxic glutaraldehyde, which was used in many studies. BSA, genipin, magnetic nanoparticle, reaction time and drug quantity were optimized. Free pemetrexed and prepared drug system were compared by monitoring the release of the drug at different pHs. Characterization studies were performed by SEM, FTIR and zeta size analysis. The synthesized nanoparticle had a hydrodynamic size of 191.6 ± 41.57 nm and a PDI of 0.340. In addition, the zeta potential value was -19.2 ± 4.37. This negative zeta potential value aids in the uptake of the nanoparticle into the cell. After all optimization studies, the drug delivery system was applied to different lung cancer cell lines (A549-luc-C8 and CRL5807) to investigate efficacy. In vitro studies have shown that this drug delivery system, which can be directed to the tumor site by applying external magnetic field, has the potential for lung cancer treatment.

Keywords: Pemetrexed, magnetic targeting, albumin nanoparticle, in vitro, controlled drug release

Acknowledgments We would like to thanks for financial support received from Ege University (Project ID: 17 FEN 005).

References

[1] Ak G.,Yılmaz H., Şanlıer Ş.,2014, Preparation of magnetically responsive albumin nanospheres and in vitro drug release studies, *Artificial Cells, Nanomedicine, and Biotechnology,* 42, USA, 10p.

[2] Futreal P. A, et al. 2001, Cancer and genomics, NATURE, Vol 409, 850-852.

[3] Zeybek A., The effect of doxorubıcın-albumın magnetıc nanopartıcles on prostate and lung cancer cells, master thesis, 2012

[4] Lemjabbar-Alaoui H., et al. Lung cancer: Biology and treatment options, 2015, *BBA*.

[5] Miklán Z., Orbán E., Bánóczia Z., and Hudecza F. New pemetrexed-peptide conjugates: synthesis, characterization and in vitro cytostatic effect on non-small cell lung carcinoma (NCI-H358) and human leukemia (HL-60) cells, 2011, J. Pept. Sci. 10.1002/psc.1407.

[6] **Dung D. T. K., et al.** Preparation and characterization of magnetic nanoparticles with chitosan coating, Journal of Physics: Conference Series **187** (2009) 012036

[7] Zhao H., et al. "Preparation of biodegradable magnetic microspheres with poly(lactic acid)-coated magnetite", Journal of Magnetism and Magnetic Materials, 2009, 321, 1356–1363.

[8] **Xu J., et al.** Preparation and Evaluation of Vancomycin-Loaded N-trimethyl Chitosan Nanoparticles, Polymers 2015, 7, 1850-1870, Doi:10.3390/polym7091488.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effect of Vitamin E and Selenium on Necrotic Cell Death in Fluorine Toxicity

Ayşe Usta[a], Veysel Yüksek[b], Sedat Çetin[c], Semiha Dede[c]

[a]*Van Yuzuncu Yil University, Department of Chemistry, Faculty of Science, Van, Turkey*
[b]*Van Yuzuncu Yil University, Ozalp Vocational High School, Van, Turkey*
[c]*Van Yuzuncu Yil University, Department of Biochemistry, Faculty of Veterinary Medicine, Van, Turkey*
ayseusta@yyu.edu.tr

Objective: The aim of this study was to investigate the effect of Vitamin E and Selenium on the necrotic pathway against sodium fluoride toxicity in the NRK-52E kidney cell line.

Material-Method: Cells were grown in optimal conditions and prepared for analysis. Proliferative concentration of vitamin E and selenium combination, ICF value of NaF was determined by MTT. In this study, control (K), fluorine (F), vitamin E + selenium (E / Se) and fluorine + vitamin E / Se (FES) groups were formed. RNA isolation and cDNA synthesis were performed 24 hours after the application of vitamin E / Se and fluorine to the cells at the specified concentrations. Expression of necrotic genes was determined by RT-PCR.

Results: The proliferation enhancing concentration of vitamin E / selenium at 24 hours (Vit E: 60 µM, Se: 0.01 µM) and the IC50 concentration of NaF (3200 µM) were found. In F and FES groups, Ripk1 expression increased by 2.7 and 5 times, Ripk3 increased by 8.3 and 5.1 times, respectively.

Conclusion: As a result, it was found that the fluoride given at IC50 concentration affects the necrotic genes studied and the highest increase occurred in Ripk3. It can be concluded that vitamin E + selenium given alone does not alter the genes much, and in the FES group, vitamin E + Se may reduce necroposis in the toxicity caused by fluorine.

Keywords: fluorine, cell culture, vitamin E, selenium, necrosis

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Cytotoxic Effects of Silica Nanoparticles and Thiol Group Functionalized Silica Nanoparticles on Human Prostate Cancer Cells

İrem Mukaddes DURMUŞ, Hatice Gül BATUR, Serdar KARAKURT

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY

iremdurmuss73@gmail.com

Prostate cancer (PCa) has a high morbidity and mortality rate among cancer types and is the second most common cancer incidence and mortality rate in men. Current treatment options such as surgery, chemotherapy, and radiotherapy have a limited role in the treatment of the disease, in addition, these methods are not as successful as other cancers in the management of PCa. Moreover, these methods have many unwanted serious side effects. So, most studies are seeking new and alternative approaches for diagnosis and treatment of cancer. The cytotoxic effects of silica nanoparticles (SiNP) have been demonstrated in both prokaryotic cells and eukaryotic cells. However, nanoparticle used has different sizes, different shapes and different surface charges is a different effect on the cells. The aim of study synthesizes SiNPs by chemical methods and modify them with thiol group (SiNP-SH) for determine the cytotoxic effects and compare of PC-3, which is one of the prostate cancer cell lines, and healthy prostate epithelial cell PNT1A. PC-3 and PNT1A growth in Ham's F12 and RPMI-1640 growth medium at 37 °C, 95% humidity and 5% CO_2 and these cells grown to be $1x10^4$ cells in 96-well plates after switching to the logarithmic phase. After 24 hours of incubation, they were treated with SiNP and SiNP-SH at varying concentrations in the range of 0-250 µM and incubated for 48 hours. Cell death and SiNP and SiNP-SH concentration were calculated from the sigmoidal graph of SiNP and SiNP-SH concentration (IC_{50}), which inhibited 50% cell growth. SiNP and SiNP-SH inhibited the PC-3 cell, which is one of the prostate cancer cells, in a dose-dependent manner and both substances showed different doses of action in the cell. In addition, in order to determine in which way SiNPs and SiNP-SHs lead PCa cells to death, controlled cell death mechanism apoptosis studies were performed with NovoCyte Flow cytometer system using Annexin V-APC and 7-AAD dyes. The effect of SiNP and SiNP-SH on protein level in the cell was realized by Western-Blot method in BAX gene. When IC_{50} values were examined, SiNP was calculated as 90 µM and SiNP-SH was calculated as 82 µM. There was no cytotoxic effect on healthy prostate epithelial cell PNT1A. When both substances were compared, both SiNP and SiNP-SH nanoparticles showed a toxic effect on the human prostate cancer cell line, and it was observed that the functionalized nanoparticle had a more toxic effect. The synthesized SiNP and SiNP-SH caused early apoptosis of the cell by 3.8-fold and 10.7-fold increase. As a result, a new approach has been presented for the fatal and high incidence of PCa treatment with SiNPs themselves and SiNP based delivery systems.

Keywords: Silica Nanoparticles, Prostate Cancer, Cytotoxicity, Protein Expression, Apoptosis

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Comparison Of Classical And Flow Cytometric Osmotic Fragility And Eosin-5-Maleimide Binding Test In The Diagnosis Of Hereditary Spherocytosis

Cemil GÜLÜM[a], Pelin EROĞLU[a], Selma ÜNAL[b], Anıl TOMBAK[c], Gülhan TEMEL[d]

[a]*Department of Chemistry, Faculty of Arts and Science, Mersin University, Mersin, Turkey*
[b]*Department of Child Health and Diseases, School of Medicine, Mersin University, Mersin, Turkey*
[c]*Department of Internal Medicine, School of Medicine, Mersin University, Mersin, Turkey*
[d]*Department of Biostatistics and Medical Informatics, School of Medicine, Mersin Uni., Mersin, Turkey*

cemilgulum@gmail.com

Hereditary spherocytosis is a type of hemolytic anemia, caused by hemolysis of erythrocytes prior to normal survival due to hereditary damage to erythrocyte membrane proteins. Disease; It is diagnosed based on family history, clinical findings, presence of spherocytes in peripheral smear and laboratory data. The most common routine laboratory test is osmotic fragility (K-OF). It has been reported that the sensitivity of the test was low and that the K-OF test with the incubated sample was more sensitive than the fresh sample. For this reason, many tests that work with different methods to replace the K-OF test are being developed. Recently, Eosin-5-maleimide binding test (EMA) and flow cytometric osmotic fragility test (FC-OF) thats based on flow cytometry method have been developed. [1-2]

In our study, it was aimed to determine and compare disease severity and cut-offs of K-OF, EMA and FC-OF tests by using both fresh and incubated samples. The statistical findings are given in Table 1.

Table 1. Statistical Findings of K-OF, EMA and FC-OF Test.

Parameters	cut-off (Threshold Value)	AUC [% 95 CI]	p	Sensitivity [% 95 CI]	Specificity [% 95 CI]
K-OF (Fresh)	≤0,5	0,935 [0,828-0,985]	<0,0001	96,67 [82,8 – 99,9]	80,0 [56,3 – 94,3]
K-OF (Incubated)	≤0,7	1,000 [0,929-1,00]	<0,0001	100,0 [88,4 – 100,0]	100,0 [83,2 – 100,0]
EMA (Fresh)	>222,64	0,897 [0,778-0,965]	<0,0001	90,0 [73,5– 97,9]	85,0 [62,1 – 96,8]
EMA (Incubated)	>193,48	0,853 [0,725-0,937]	<0,0001	93,33 [77,9– 99,2]	75,0 [50,9 – 91,3]
FC-OF (Fresh)	>67,29	1,000 [0,929-1,000]	<0,0001	100,0 [88,4– 100,0]	100,0 [83,2 – 100,0]
FC-OF (Incubated)	>7,95	0,930 [0,821-0,983]	<0,0001	80 [61,4-92,3]	95 [75,1-99,9]

When the success of parameters in classification were compared, K-OF (Incubated)-EMA (Fresh) ($p = 0.0397$), K-OF (Incubated)-EMA (Incubated) ($p = 0.0211$), EMA (Fresh)-FC-OF (Fresh) ($p = 0.0397$) and EMA (Incubated)-FC-OF (Fresh) ($p = 0.0211$) were statistically significant, but there were no statistically significant difference in terms of other parameters.

Keywords: Hereditary spherocytosis, Flow cytometric osmotic fragility test, Eozin-5-Maleimid, EMA binding test, Osmotic fragility, Flow cytometry

References:

Won, D., L., Suh, J., S., Flow Cytometric Detection of Erythrocyte Osmotic Fragility. *Clinical Cytometry,* **2009**, *76B:135-141.*

Ciepiela, O., Old and new insights into the diagnosis of hereditary spherocytosis. *Ann Transl Med,* **2018**, *6 (17):339.*

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Antibacterial Activities of Extracts of *Lavandula*, *Ribes* and *Mentha* Against Oral Bacteria and Its Non-enzymatic Antioxidant Activities

Gulten Okmen, Busra Eroglu, Rıdvan Tekin, Kutbettin Arslan, Neslihan Balpinar, Irem Camur

Mugla Sitki Kocman University, Faculty of Science, Department of Biology, Turkey

bussraeroglu@gmail.com

ABSTRACT

There is an increasing interest in medicinal plants as a natural alternative to synthetic drugs, particularly against microbial agents because of the evergrowing problem of antibiotic resistance. Several reports have been published in recent years on the antimicrobial activity of some extracts derived from plants: activity against important bacteria was scientifically confirmed. *Lavandula angustifolia* (Labiateae) is commonly used in cosmetic and therapeutic applications. Within aromatherapy and wellness industries, the oil has been indicated for the treatment of conditions, such as rhinitis, coughs, minor burns, and in treatment of wounds. The well-known and widely used peppermint (*Mentha piperita* L.) (Labiateae) is a cultivated natural hybrid of *Mentha aquatica* L. and *Mentha spicata* L. Although a native genus of the Mediterranean region, it is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. *Ribes nigrum* L. (Grossulariaceae), is a woody shrub spontaneously growing in central and eastern Europe, while in temperate regions it is mostly cultivated. *Ribes nigrum* leaves are used in European folk medicine to treat rheumatism, arthritis and respiratory problems.

The aim of this study was to test the plant extracts against oral bacteria. The purpose of this was to create directly comparable, quantitative, antimicrobial data and to generate data for extracts for which little data exist. The extracts were showed different inhibition zones against bacteria. In this study, methanol, ethanol and water were used as solvents. The methanol extract of *Lavandula* showed highest inhibition zone against oral pathogen MBKK5, and the zone was 14 mm. The lowest MIC value is 6500 µg/ml. In addition, the extracts were tested against the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radicals for antioxidant activity. As a results, the extracts of plants have antibacterial, and antioxidant potential.

Key words: *Mentha piperita*, *Lavandula angustifolia*, *Ribes nigrum*, oral bacteria, antibacterial activity, antioxidant activity

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Antibacterial Activities Against Mastitis Pathogens of *Punica granatum* L. Flowers and Its DPPH Scavenging Activity

Gulten Okmen, Rıdvan Tekin, Busra Eroglu, Kutbettin Arslan, Neslihan Balpinar

Mugla Sitki Kocman University, Faculty of Science, Department of Biology, Turkey

bussraeroglu@gmail.com

ABSTRACT

Economically, mastitis is the most important and costly disease of dairy herds. Antibiotics are widely used in the treatment of the disease. However, this widespread use of antibiotics causes both antibiotic residues in milks and antibiotic resistance developed in bacteria. Recently, researches are focused on discovering and using new antibiotics against these bacteria. Plants are screened for this purpose. Lythraceae family consists of single or perennial herbaceous, shrubs or trees. Since the family members are used as dye plants, ornamental plants and medicinal plants in the industry and they have a high economic value. The aim of this work was to investigate the antibacterial effects of *Punica granatum* extracts against mastitis pathogens, and its antioxidant potentials. The antibacterial activity test was done by Kirby-Bauer method. *Punica granatum* was collected from three region. These are included Mugla, Denizli and Isparta from Turkey. The extracts of Mugla showed maximum inhibition zone against a lot of bacteria. The methanol extract showed highest inhibition zone against Coagulase-negative *Staphylococci*- 37 (CNS-37), and the zone was 22 mm. The lowest MIC value is 6500 µg/ml. In addition, the extracts were tested against the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radicals for antioxidant activity. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was chosen as a standard antioxidant. The extracts of *Punica granatum* have antibacterial, and antioxidant potential.

Key words: *Punica granatum*, mastitis, antibacterial activity, antioxidant activity

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Neural Cellular Effects of the Gold Nanomaterials with Different Surface Functionalities and Physical Properties

Ilyas Ozcicek[a, b*], Nese Altuncu[a, b], Cagri Cakici[c]

[a]*Department of Medical Biology, School of Medicine, Istanbul Medipol University, Turkey*
[b]*Regenerative and Restorative Medicine Research Center, Istanbul Medipol University, Turkey*
[c]*Department of Medical Biochemistry, School of Medicine, Istanbul Medipol University, Turkey*
iozcicek@medipol.edu.tr

Gold nanoparticles and gold nanorods are metallic nanomaterials which have unique physical and chemical properties, have a wide range of uses in biomedical fields, electronics, chemistry, medicine and pharmacy. They are often preferred for cell and tissue imaging, RNA/drug delivery, targeting studies, photothermal therapy and biosensors. The method of the synthesis, shape, material size, surface modification, cell type, exposure duration and concentration of the nanomaterials play a decisive role on the nanotoxicity and theranostic studies[1, 2]. Many neurodegenerative diseases and syndromes can be diagnosed and treated by transferring nanoteranostatic materials into the neural cells and specifically into the nucleus at adequate and effective levels.

In this study, highly monodisperse two different size groups of gold nanoparticles ($AuNP_{20}$ and $AuNP_{50}$) and stable gold nanorods (AuNRs) were synthesized by using seeding growth method. Synthesized gold nanomaterials were electrostatically modified by polyethyleneimine (PEI) and polyethylene glycol (PEG). DRG primary sensory neurons were isolated from BALB/c mice. Using gold nanomaterials groups which have three different concentrations (1 µg ml^{-1}, 10 µg ml^{-1}, 100 µg ml^{-1}), neural cell viability, apoptosis and general oxidative stress levels were quantified. Cellular uptake of the gold nanomaterials were quantified by using ICP-MS instrument.

Using seeding growth method, highly monodisperse PEI/PEG coated AuNPs and stable AuNRs were synthesized. Especially in the gold nanorod groups, surface functionalizations have increased cellular viability at a high level. The results of the apoptosis analysis coincided with the cell viability results. For the gold nanomaterial groups, additional surface coating generally reduced the production of reactive oxygen species (ROS). At the same time, ICP-MS analysis results showed that cellular uptake amounts of the gold were significantly higher for surface functionalized gold nanomaterial groups.

Surface functionalization processes provide superior properties to the gold nanomaterials in terms of nanotoxicological parameters and the potential teranostic applications in the future studies.

“This study was supported by The Scientific and Technological Research Council of Turkey (Grant no: 217S135).”

References:

Khlebtsov, N. and Dykman L., Chem Soc Rev., 40(3), 1647-1671, 2011.
Wang, X., et al., Journal of Nanoparticle Research, 15(9), 2013.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Chromium Tolerance and Biosorption in Cyanobacteria Isolated from Paddy Fields

Ender BOZANTA, Gulten OKMEN, Kutbettin ARSLAN

Mugla Sitki Kocman University, Faculty of Science, Department of Biology, Mugla, TURKEY

gultenokmen@gmail.com

The present study aimed to investigate the effects of various chromium concentrations (1.25 mg/L to 80 mg/L) on chlorophyll-a, total carbohydrate and total protein contents of 4 cyanobacterial species. Cyanobacterial species isolated from paddy fields. Chlorophyll-a, protein and carbohydrate content in all of cyanobacterial species were measured by spectrophotometric method. The physiological parameters of *Anabaena* sp. GO4 and GO6 were stimulated at lower chromium concentrations (1.25- 2.5 mg/L). But, in the high concentrations of chromium are more than 20 mg/L, chlorophyll- *a* and other parameters of all cultures were completely reduced. In biosorption studies, influence of varying the conditions for removal of metal ions, such as the dosage of biosorbent (0.16 g), pH of aqueous solution (pH 1 for chromium), initial metal ion concentrations (20 mg/L), the temperature (35 °C) and agitation speed were investigated. The best metal removal performance was observed on *Gloeothece* sp. GO9. Fourier Transform Infra Red (FT-IR) analysis of the biomass exposed to chromium indicates that amino and carboxylate groups in the biomass are involved in biosorption process. This study demonstrated that the biomass of *Gloeothece* sp. GO9 could be used as an efficient biosorbent for the treatment of chromium (VI) bearing wastewater.

Key words: Cyanobacteria, Chromium, Chlorophyll, Total Carbohydrate, Total Protein, Biosorption

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Possible nerve injuries in the course of Total Mesorectal Excision

Azimov E.H.

Azerbaijan Medical University, I Department of Surgical Diseases

elman-azimov@mail.ru

Introduction. In the course of TME, both laparoscopic operations and the ability of the vegetative nerve elements to detect both conscious and unconscious (for the protection of oncological principles) are likely. Damage occurs both in sympathetic fibers and parasympathetic fibers, which ultimately leads to urogenital disorders.

Material and methods of the study. Observations were performed based on the diagnosis and treatment of 145 patients diagnosed with flat gastric cancer and endoscopic polyps. Patients are divided into two groups: 1) laparoscopic group - 69 patients 2) open group 76 patients. Clinical examination of patients, general analysis of blood and urine, blood biochemical analysis, CT scan of breast and abdominal cavity, MRT of small bowel, R-epophilia of the chest, colonoscopy (biopsy), definition of oncomers in the blood (CEA, CA 19-9) p. inspections were carried out. Nerve injuries were examined as a result of surgical and postoperative clinical analyzes (7 days and 6 months postoperatively).

Discussion of the research. According to literature data and personal observations, anatomical and clinical classification of nerve injuries during TMJ was performed. The damage and the severity of the lesions were attempted to interpret every damaged clinical symptom as NI-1, NI-2, NI-3, NI-4 and NI-5. For investigate urogenital functions at patients who have nerve injuries(NI+) is used EFII and MVS. There is statistical difference between laparoscopic groups and open groups. EFII at first month at laparoscopic group is 41,8±14,7, at open group is 39,7±15,3,at NI+ group indicators are 56,2±12,8 and 55,3±11,8,at 6 months indicators are (NI-)41,3±16,4 and 40,6±19,9,at NI+ groups indicators are 42,6±21,5 and 40,6±19,9. MVS at NI- laparoscopic group at first week is 16,4±3,2,at open group is 16,2±3,1,at sixth month MVS is according to 17,5±2,8 and 17,6±2,7

Result. We believe that the classification reflecting nerve damage in TME will pay great attention to the theoretical and practical point of view, will be one of the additional guiding forces during operation. Thus, nerve injuries occurring during TME influence on urogenital functions [1].

References

1. Zhang C., Li G.X., Ding Z.H. et all. Preservation of the autonomic nerve in rectal cancer surgery, anatomical factors in ligation of the inferior mezenteric artery , 2006, 49-52.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Integrated Multi-Omics Approaches for Better Understanding of Phenotype

Cemil Can Eylem, Sedef Kır, Emirhan Nemutlu

[1]*Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry*

[2]*Hacettepe University, Faculty of Pharmacy, Department of Biochemistry*

Sıhhiye, Ankara, TURKEY

[*]E-mail: enemutlu@hacettepe.edu.tr

Living cells represent an integrated and interacting network of genes, transcripts, proteins, small signaling molecules, and metabolites that define cellular phenotype and function. Traditionally the focus of biomedical research was on individual genes, single protein targets, single metabolites, and metabolic or signaling pathways. This “molecular reductionist” paradigm was based on the assumption that identifying genetic variations and molecular components would lead to discovery of cures for human diseases. However, most of diseases are complex and multi-factorial and the disease phenotype is determined by the alterations of multiple genes, pathways, proteins and metabolites (at cellular, tissue, and organismal levels). Therefore, an integrated "omics" approach is more viable direction for uncovering alterations in metabolic networks, disease mechanisms, and mechanisms of drug effects [1,2].

Technological innovations and translation of basic discoveries to clinical practice drive advances in medicine. Today's innovative technologies enable comprehensive screening of the genome, transcriptome, proteome, and metabolome. The detailed knowledge, converged in the integrated "omics" (genomics, transcriptomics, proteomics, and metabolomics), holds an immense potential for understanding mechanism of diseases, facilitating their early diagnostics, selecting personalized therapeutic strategies, and assessing their effectiveness. Metabolomics is the newest "omics" approach aimed to analyze large metabolite pools indicators of energy and metabolic imbalances like the ones created by genetic deficiencies, myocardial ischemia, heart failure, inflammatory and neurodegenerative disorders, etc. The analysis of metabolic fingerprints left by disease processes and metabolic monitoring of disease progression or treatment efficacy plays a crucial role in personalized and predictive medicine. Therefore, bringing new metabolomics technologies and its integration with other omics platforms will let better understanding of system biology [3,4]. In this presentation, we will give a couple of examples to show the benefits of integrated omics platforms especially metabolomics data with the other omics data on disease or plant system biology.

Reutens AT, Individualized therapy for diabetes mellitus—just a promise? *Nature Reviews Endocrinology,* 6, 426-427, 2010.

Nemutlu E, Zhang S, Gupta A, Juranic NO, Macura S, Terzic A, Jahangir A, Dzeja P, Dynamic phosphometabolomic profiling of human tissues and transgenic models by 18O-assisted 31P NMR and mass spectrometry. *Physiological Genomics*, 386-402, 2012

E.M. Gonulalan, E. Nemutlu, O. Bayazeid, E. Koçak, F.N. Yalçın, L.O. Demirezer, Metabolomics and Proteomics Profiles of Some Medicinal Plants and Correlation with BDNF Activity**,** Phytomedicine**,** 2019

E. Nemutlu, G. Orgul, T. Reçber, E. Özkan, E. Aydın, E. Özkan, M. Turgal, M. Alikaşifoğlu, S. Kır, M.S. Beksaç, Metabolic Infrastructure of Pregnant Women with Trisomy 21 Fetuses; Metabolomic Analysis, Zeitschrift für Geburtshilfe und Neonatologie, 2019

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Hemorheometer-On-Chip: Microfluidic Analysis of Whole Blood Flow Parameters

Ziya Isiksacan, Murat Serhatlioglu, Caglar Elbuken

National Nanotechnology Research Center (UNAM) and Institute of Materials Science and Nanotechnology, Bilkent University, Ankara, Turkey

elbuken@unam.bilkent.edu.tr

Whole blood flow dynamics determine the tissue oxygenation levels, hence carry life-critical value in several pathological conditions. Despite the long history of research on blood, the state-of-the-art techniques lack the ability to determine hemorheological properties of blood in a clinical setting[1]. For this purpose, we developed a method to determine flow dynamics of blood using an optical-based detection technique[2]. The system uses a novel pumping mechanism to induce in-vivo like flow conditions in a microfluidic channel that requires only a drop of whole blood for analysis. The measurement is completed in three minutes using a handheld analyzer device and can determine hemorheological properties, which can be linked to diseases related to red blood cell anomalies. Unlike the systems that are based on experimental single cell investigation or computational flow dynamics, these results open the avenue for blood studies that capture the cell-cell interactions as well as cell-medium interactions as in the circulatory system[3]. The non-Newtonian characteristics of the red blood cells and the medium form the basis of the measurement[4], which may also contribute to the biochemical analysis of whole blood used in several other systems.

References:

A. L. Copley and G. V. F. Seaman, Clin. Hemorheol. Microcirc., 2016, 1, 117–119

Z. Isiksacan, O. Erel and C. Elbuken, Lab Chip, 2016, 16, 4682–4690

J. Duez, M. Carucci, I. Garcia-Barbazan, M. Corral, O. Perez, J. L. Presa, B. Henry, C. Roussel, P. A. Ndour, N. B. Rosa, L. Sanz, F. J. Gamo and P. Buffet, Nat. Protoc., 2018, 13, 1362-1376

G. Gompper, F. Nicoud, D. A. Fedosov, S. Mendez, J.-M. Fromental, L. Lanotte, V. Claveria, J. Mauer and M. Abkarian, Proc. Natl. Acad. Sci., 2016, 113, 13289–13294

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Assessment of the physicochemical features of boric acid-modified human hair keratin hybrid mineral/polymeric structures for bone tissue engineering

Fatma CAL and Yavuz Emre ARSLAN

Canakkale Onsekiz Mart University, Faculty of Engineering, Department of Bioengineering, Regenerative Biomaterials Laboratory, 17100, Canakkale, Turkey
calfatma17@gmail.com

Nowadays, the main goal of bone tissue engineering in biomaterial design is to construct protein-based bioscaffolds that facilitate regeneration and provide the integration in native bone tissue. Similar to many other natural polymers, keratin has unique physicochemical and biological characteristics such as good mechanical properties, cellular biocompatibility, excellent biological activity, and so on. Due to its wound healing and regenerative capacity, keratin, which extracted from human hair, has been used in the production of osteoconductive bioscaffolds in this study. Furthermore, the composites composed of keratin and biologically active minerals are thought to be an important phenomenon in the fabrication of bioscaffolds with desired properties. As clearly indicated in the literature, the boron mineral has a significant effect on bone development, wound healing and immune system. In addition to this, it has also been shown that the expression levels of the genes involved in mineralization of osteoblasts are increased as well. From this aspect, human hair keratin has been chemically combined with boron molecules to promote bone tissue regeneration. The boron modification of the keratin bioscaffolds has been conducted under mild conditions with boric acid. The morphological structure of the boron-modified human hair keratin scaffold has been examined on a stereomicroscope. The changes on the surface morphology were deeply investigated by scanning electron microscopy. Besides, energy dispersive spectroscopy (EDS) analysis has been performed to determine the percentage of the boron inside the bioscaffold. The newly formed chemical bonds due to keratin and boric acid reaction was proven by attenuated total reflectance-Fourier transform infrared spectroscopy analysis. As a result, human hair keratin-boric acid hybrid bioscaffolds, which possess a highly porous and durable structure, have been successfully fabricated. We believe that further studies are necessitated to ascertain the biocompatibility and osteoconductive/inductive properties of prepared scaffolds. The authors gratefully acknowledge financial support from Canakkale Onsekiz Mart University, Scientific Research Projects Coordination Unit (Project ID. FYL-2019-2955).

Keywords: Human hair keratin; boron, bioscaffold; bone tissue engineering.

2.EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

ANALYSES and MODELING of OVARIAN CANCER MICROARRAY DATA

BARIŞ SU KARAKELLE

Bilkent 2 H Blok no : 23 Çankaya ANKARA
e171636@metu.edu.tr

Ovarian cancer is one of the common cancer types among other oncological diseases. The major causes of this cancer can be listed as age, obesity, hormone therapy, material inheritance and contraceptive pills. Due to its generality and importance, many researches have been conducted from distinct labs about this illness and its plausible causes have been intensively investigated either in microarray studies, where just part of the related genes are detected, or in the pairwise correlation analyses between the disease and selected symptoms via contingency tables. Hereby, in this study, we aim to combine these different sources of data under a single network model in such a way that the microarray studies conducted for ovarian cancers will be merged by performing specific normalization approaches. Then the differentially expressed genes will be clustered and described by complex biological network methods. For the modelling, we propose MARS (multivariate adaptive regression splines) and GGM (Gaussian graphical model) among alternatives. Finally, the biological results will be interpreted by related literature. We also consider to use our findings in a more complex model where the other causes of the disease are included in an ovarian cancer-diagnosis model.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Photothermal Antibacterial Activity of Black Phosphorus Based Nanohybrids against a Nosocomial Pathogen *Enterococcus faecalis*

Ilknur AKSOY[a], Huseyin KUCUKKECECI[b], Fatih SEVGI[c], Onder METIN[d], Imren HATAY PATIR[a]

[a]*Selçuk University, Faculty of Science, Department of Biotechnology, 42031, Konya, Turkey*

[b]*Koç University, Materials Science and Engineering, 34450, Istanbul, Turkey*

[c]*Selçuk University, Vocational School of Health Services, Department of Medical Services and Techniques, 2031, Konya, Turkey*

[d]*Koç University, Faculty of Science, Department of Chemistry, 34450, İstanbul, Turkey*

İlknuraksoy1@gmail.com

Recently, 2D semiconductor-based materials have been evolved to kill pathogen microorganisms in exposure NIR (Near Infrared) light irradiation.[1] Nowadays, 2D Black Phosphorus has gained attention in biotechnology, materials science and biomedical fields owing to its unique layered structure, biodegradable and non-toxic properties. Besides, the tunable band gap feature of exfoliated BP enables them with distinguished photocatalytic, optoelectronic and antibacterial activities under NIR light.[2] Herein, exfoliated BP were conjugated with Au nanoparticles (AuBP) used as NIR light-driven antibacterial agent against a nosocomial pathogen *Enterococcus faecalis*. The photothermal effect of exfoliated BP grants AuBP nanohybrids immediately degrade bacterial membrane in exposure NIR light irradiation. Moreover, by means of inert nature of Au nanoparticles, they have also been used to optimise 2D exfoliated BP to enhance their antibacterial activity in curative studies.[3] Accordingly, in this work to prove antibacterial efficiency of AuBP nanohybrids under NIR light irradiation, 24 hours Optical Density (OD) measurement and agar plate experiments were carried out. The results demonstrate that neither NIR light nor exfoliated BP in dark has apparent impact on *E. faecalis* growth. Suprisingly, when AuBP were exposured with NIR, the bacterial growth completely suppressed, indicating excellent antibacterial properties of AuBP nanohybrids under NIR light irradiation.

1. Yin, Q., et al., *Plasmonic molybdenum oxide nanosheets supported silver nanocubes for enhanced near-infrared antibacterial activity: Synergism of photothermal effect, silver release and photocatalytic reactions.* Applied Catalysis B: Environmental, 2018. **224**: p. 671-680.
2. Ouyang, J., et al., *A black phosphorus based synergistic antibacterial platform against drug resistant bacteria.* Journal of Materials Chemistry B, 2018. **6**(39): p. 6302-6310.
3. Wu, Q., et al., *Development of functional black phosphorus nanosheets with remarkable catalytic and antibacterial performance.* Nanoscale, 2018. **10**(22): p. 10428-10435.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Biosynthesis of Ag-Cu Bimetallic Nanoparticle using Aqoues Extract of Dried Husk of Walnut (*Juglans regia*) And Determination of Its Antimicrobial Properties

Sona Ayadi Hassan[1], Parinaz Ghadam[1], Ahya Abdi Ali[2]

Department of Biotechnology, Faculty of biological sciences, Alzahra university, Vanak, Tehran, Iran

Department of Microbiology, Faculty of biological sciences, Alzahra university, Vanak, Tehran, Iran

pghadam@alzahra.ac.ir

Abstract

Recently, versatile applications have been reported for a broad spectrum of the nanoparticles. The desire for the green synthesis of nanoparticles by natural sources is on the rise expotentially, due to easy, rapid and environmentally-friendly nature of the process. In addition, the fabrication of bimetallic nanoparticles with unique applications in electronics, catalysis, imaging etc., has attracted attentions of many researchers toward itself worldwide. The reason for this tendency errupts out of the fact that these compounds are composed of two metals with two distinct or cooperative features, leading to new or elevated properties. Up to now, there have been numerous reports which are alarming us for the emergence of multi-drug resistantant bacteria and have triggered new studies in this aspect. Consequently, there have been many remarkable results indicating for the potential antimicrobial effects of nanoparticles, especially biogenic silver nanoparticles and its derivatives. In the present study, Ag-Cu bimetallic nanoparticles were biosynthesized at ambient and green condition by adding the aqueous extract of dried *Juglans regia* green husk into 1mM AgNO3 and 1mM Cu(CH3COO)2 solution simultaneously and the antimicrobial tests were carried out to evaluate its potential against some standard and multi-drug resistant bacteria. As the production of the bimetallic nanoparticles progressed, the color of the sample changed from glassy brown to smoky brown, indicating the formation of Ag-Cu bimetallic nanoparticles initially. In order to detect the physiochemical features of the nanoparticle, various methods such as UV-Visible spectroscopy, dynamic light scattering (DLS), energy dispersive X-ray (EDX) and X-ray diffraction (XRD) were exploited. The electromagnetic absorbance of the Ag-Cu bimetallic nanoparticles appeared at 511 nm. The monodisperse nanoparticles had hydrodynamic diameter close to 86.77 nm. Furthermore, the results of XRD revealed that the bionanofabricated nanoparticles are amorphous compounds. The equal presence of both silver and copper in the structure of Ag-Cu bimetallic nanoparticles was determined by EDX, addressing the formation of a typical bimetallic nanoparticle. Followingly, the effect of Ag-Cu bimetallic nanoparticle on some standard and MDR bacteria was examined.

Keywords: Bimetallic Nanoparticles, *Juglans regia*, antimicrobial effects

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Protective Role of Thioredoxin- Interacting Protein (TXNIP) Against Oxidative Stress in Mouse Heart Tissue.

Sevda ALTUN[a,b], Harun BUDAK[a]
[a]*Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Turkey*
[b]*Department of Bioengineering, Faculty of Engineering, Ege University, Izmir, Turkey*
sevdambg2012@gmail.com

Although iron is an important bioelement and required for many vital biological processes, its deficiency or excess can lead to significant clinical pathologies[1]. Excess iron in the body causes oxidative stress by increasing the production of reactive oxygen species (ROS) which is the underlying factor in many cardiovascular diseases[1,2]. Thus, the response of the antioxidant defense system to maintain redox balance is very important to protect the heart tissue during the oxidative stress[2]. The redox-related protein complex thioredoxin- interacting protein (TXNIP) is a critical regulator for ROS signaling and plays a role in the pathogenesis of various diseases[2]. Since the increased TXNIP expression by histone acetylation is known to cause cardiac dysfunction epigenetic downregulation of Txnip in cardiovascular diseases may offer novel therapeutic approaches[2]. Here, the effect of iron on TXNIP was investigated at the gene and protein levels in mouse heart tissues. For this purpose, 10 male BALB/c mice (3 months old) were divided into 2 groups. Control group was intraperitoneally injected with 0.5 mg of dextran 5 solution. In the treatment group, 5 mg iron dextran solution was intraperitoneally injected twice weekly for 3 weeks to form systemic iron overload. The quantitative iron content and the amount of glutathione (GSH), which is oxidative stress markers were spectroscopically measured. Then, the quantitative gene expression changes of *Tip60* which have histone acetyltransferase (HATs) activity and also *Txnip* expression were examined by Real-Time PCR. The impact of the gene expression on quantitative protein expression was demonstrated by western blot. According to our results, no change was observed in the GSH level. While *Txnip* gen expression level was not changed with iron overload in the mouse heart, there was a significant decrease in TXNIP protein expression. Furthermore, gene expression of *Tip60* was decreased with iron overload. In conclusion, we reported here that the gene expression of *Txnip* did not correlate to protein expression, and the actual effect of iron overload on the TXNIP is observed at the protein level. It is thought that the decreased TXNIP protein expression may protect the heart tissue against oxidative stress by Tip60 mediated epigenetic regulation. This hypothesis should be elaborated with further studies.

Keywords: Heart, Iron metabolism, Oxidative stress, TXNIP, Epigenetic.

Gozzelino, R. and Arosio, P. Iron homeostasis in health and disease. International journal of molecular sciences, 2016, 17 (1), 130.

Bompada, Pradeep, et al. Histone acetylation of glucose-induced thioredoxin-interacting protein gene expression in pancreatic islets. The international journal of biochemistry & cell biology, 2016, 81: 82-91.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

A newly designed physical construct from Si-incorporated human hair keratin for regenerative medicine applications

Fatma CAL[a], Tugba SEZGIN ARSLAN[b], Ugur CENGIZ[c], and **Yavuz Emre ARSLAN[a]**

[a]*Canakkale Onsekiz Mart University, Faculty of Engineering, Department of Bioengineering, Regenerative Biomaterials Laboratory, 17100, Canakkale, Turkey*
[b]*Ankara University, Science Faculty, Department of Chemistry, [b]Bioelectrochemistry Laboratory, Tandogan, 06100 Ankara, Turkey*
[c]*Canakkale Onsekiz Mart University, Faculty of Engineering, Department of Chemical Engineering, 17100, Canakkale, Turkey*
yavuzea@gmail.com

The human body itself could be re-defined as a continuous 'biomaterial manufacturing center' for the scientists who deal with tissue engineering problems. With this in mind, the relationship between the human body and the materials (e.g., natural or synthetic polymers, minerals) used to evolve an artificial organ, or tissue is an important issue. In this study, human hair keratin has been selected as a natural biomaterial source to fabricate a bioscaffold for bone regeneration. Human hair as a waste material has been thus recovered by a recycling process for bone tissue engineering applications. According to the previous studies, the human hair-derived keratin performs superior features like being biocompatible, biodegradable, and revealing an excellent hosting without any immune reaction while transplantation. Those fascinating properties of keratin have been combined with silica, one of the inorganic bioactive minerals, to promote biofunctionality for neo bone formation. The incorporation of silica molecules into keratin chains has been fulfilled with the sol-gel technique using tetraethyl orthosilicate (TEOS) solution as a silica precursor. The Si-modified human keratin structure was characterized using FTIR and ^{1}H-NMR analyses for proving the silanization process. The morphology of the bioscaffold was investigated by scanning electron microscopy-energy dispersive spectroscopy (SEM-EDS) analysis. In addition to this, the thermal properties of the prepared scaffolds were examined in-dept via differential calorimetry scanning (DSC) and thermogravimetric analysis (TGA). The SEM-EDS analysis demonstrated that the silica content was 12.90% of the total bioscaffold mass. The SEM micrographs revealed the silanized-keratin bioscaffolds to be possessed a unique interconnected porous morphology, which is required for cell hosting and growth. The TGA results indicated that 83.17% of the total mass was lost at 333.79°C (named as T degradation point), which are also in close agreement with the DSC analysis data. As a result, we believe that the novel bioscaffold fabricated by the silanization of keratin could have the potential to regenerate of damaged bone tissue.

Keywords: Human hair keratin; hydrogel; TEOS; sol-gel; bioscaffold; bone tissue engineering.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Determination of capsaicinoids in chili peppers by soxhlet extraction-high performance liquid chromatography

Sezgin Gündüz[a], Miraç Tüysüz[a], Erdoğan Özgür[a,b], Canan Armutcu[a], Mehmet Emin Çorman[c], Lokman Uzun[a,b]

[a]*Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, Türkiye*
[b]*Hacettepe University, Advanced Technologies Application and Research Center, Ankara, Türkiy*
[c]*Sinop University, Faculty of Sciences and Arts, Department of Chemistry, Sinop, Turkey*
(mehmetemincorman@gmail.com)

Capsaicinoids are a group of pungent chemical analogues which are usually found in chili peppers.[1] Capsaicinoids main characteristic is specified binding to pain and heat receptors. Capsaicin is the most abundant and potent analogues in peppers. It has anti-mutagenic and anti-carcinogenic (properties, and has analgesic, and anti-inflammatory effects.[2] Due to properties and current application in the medical area as pharmaceuticals, and in defensive sprays capsaicinoid compounds have been widely studied and for this purpose diverse procedures have been reported for the isolation and analysis of these secondary metabolites.

In this study, the capsaicin was exctracted from red pepper by soxhlet method and then analyzed by HPLC., In order to characterize capsaicin in chili pepper, optimum extraction parameters such as solvent type, temperature and time were investigated using soxhlet extraction as the pre-concentration step. After the completed extraction, capsaicinodis extracts were analysed by high performance liqud chromatography. We have evaluated an alternative mode for capsaicin of two-dimensional (2D) HPLC in the reversed-phase liquid chromatography (RPLC) mode using monolith columns at first dimension (1st-D) and second dimension (2nd-D).

**This study is supported by Hacettepe University Research Fund Project, Project No: 17742.*

References:

Berbero, G.F.; Barraso, C.G.. *Talanta.* **2008**, *1332*-1337.
Bajer, T; Ventura, K. *J. Food. Compos. Anal*. **2015**, 40, 32-38

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Computational Construction of *Neisseria Meningiditis* Fe^{3+} Transfer Triple Complex

Mehmet Özbil[a], *Gizem Nur Duran*[b]

[a]*İstanbul Arel University, Dept. of Molecular Biology and Genetics, Büyükçekmece, İstanbul, TURKEY*

[b]*Marmara University, Dept. of Chemistry, Kadikoy, İstanbul, TURKEY*

email: mehmetozbil@arel.edu.tr

Gram-negative bacteria Neisseria meningiditis is responsible for meningitis and gonorrhea. According to World Health Organization (WHO) in 2014 alone 11.908 people were affected and 1.146 people died in Africa.[1] The bacteria needs Fe^{3+} ion for its survival. Thus it acquires the ion from human cells. Fe^{3+} ion transfer is utilized by transferrin binding proteins Tbp-A and Tbp-B. Extracellular protein Tbp-B binds to human transferrin (hTF) and this complex bind to membrane protein Tbp-A for the formation of Tbp-A-Tbp-B-hTF triple protein complex.

In 2012 Noinaj et al.[2] revealed an x-ray structure of Tbp-A and Tbp-B alone, and also Tbp-A-hTF protein complex. In this study researchers proposed Fe^{3+} ion transfer mechanism, where upon complexation hTF C -lobe bound Fe^{3+} ion is loosened from its coordination shell formed by Asp392, Tyr426, Tyr517, His585 amino acid residues and a bicarbonate ion. Fe3+ ion is then transferred through the beta barrel ion channel of Tbp-A, into the intracellular space. However, this proposal was made on the dublex complex.

To date there is no 3-D structure of Tbp-A-Tbp-B-hTF triple complex. Lack of this structure prohibits the conformation of this transfer mechanism. The aim of this study is to build the triple complex through series of individual 100 ns classical molecular dynamics (MD) simulations. Extracellular space proteins Tbp-B and hTF were placed into cubic water filled MD simulation box and Tbp-A was embedded into DPPC lipid membrane model. Then, the most representative structures of proteins from each simulation were taken and by applying protein-protein docking simulations Tbp-A-Tbp-B-hTF triple complex was built. This structure enabled us to reveal molecular interactions that enable complexation at the molecular level. In the next step virtual screening of small molecules to disrupt these interaction interfaces will take place.

References :

Meningococcal meningitis. 2016; Available from: http://www.who.int/mediacentre/factsheets/fs141/en/.

Noinaj, N., Easley N.C., Oke, M., Mizuna, N. et al., *Neisseria.* Nature, **2012**, 483, 53.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Analysis of Synergic Anti-Canser Effects of Gambogic Acid and Trastuzumab on HER2 Positive Breast Cancer

Ahmet ÇETİN, Murat ÇANKAYA

Department of Biology, Faculty of Sciences and Art, Erzincan Binali Yıldırım University

ahmetcetin06@gmail.com

Cancer is one of the biggest health problems in the world, causing millions of deaths every year. The most common cancer in women is breast cancer. One of the breast cancer types in the molecular classification is HER2 positive breast cancer with overexpression of the Human Epidermal Growth Factor Receptor-2 (HER2) gene.

Drugs that are most frequently transferred to clinical applications and produced for therapy among cancer immunotherapy methods are Monoclonal antibodies[1]. Trastuzumab is a monoclonal antibody targeting HER2. This monoclonal antibody binds to HER2 and inhibits the signaling pathways activated by the HER2 receptor in cancer cells. Gambogic acid ($C_{38}H_{44}O_8$) is the main active compound of gamboge released from *Garcinia hanburryi* tree[2]. Pharmacological studies over the last half-century have shown that gambogic acid has potent anti-tumor activity against many types of cancer, including breast cancer[3].

In the treatment of cancer, studies on the creation of a synergistic effect in combination with another cytotoxic agent to obtain more effective results from monotherapy are quite common. In this study, we examined the synergistic anticancer effect of gambogic acid and Trastuzumab on HER2 positive breast cancer. MDA-MB-453 with HER2 receptor expression was used as cell line. Firstly, synergistic effect studies were performed on MDA-MB-453 cell line and then the combination index (CI) values of the results were calculated. Synergistic effect observed (5µM/50µM). Finally, expression and protein analysis of Caspase-9, Bax and Her-2 genes were performed.

References

Waldmann, T. A. (2003) "Immunotherapy: past, present and future", ***Nature medicine***, 9 (3), 269.

Auterhoff, H., Frauendorf, H., Liesenklas, W., Schwandt, C. (1962) "The chief constituents of gamboge resins. 1. Chemistry of gamboge", ***Archiv der Pharmazie und Berichte der Deutschen Pharmazeutischen Gesellschaft***, 295 833-846.

Wang, X., Chen, W. (2012) "Gambogic acid is a novel anti-cancer agent that inhibits cell proliferation, angiogenesis and metastasis", ***Anti-cancer agents in medicinal chemistry***, 12 (8), 994-1000.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Bootstrap-based Model Selection Criteria in Biological Networks

Mehmet Ali Kaygusuz[a], Vilda Purutçuoğlu[a]

[a] *Department of Statistics, Middle East Technica University, Ankara, Turkey*

malik_8198@hotmail.com, vpurutcu@metu.edu.tr

In the structure of the protein-protein interaction networks, due to the high dimension of systems p regarding the number of observations per gene n, i.e., $n<<p$, and the sparcity of interactions as well as the high correlation between genes, the estimation of the systems becomes challenging and thereby, the best fitted mathematical model, which can explain the biological activation, turns to be a model selection problem. Accordingly, the selection of the optimal model among alternates is done via either classical model selection criteria such as Akaike's information criterion (AIC) and Bayesian information criterion (BIC) or some specific methods suggested for sparse biological networks such as extended BIC (eBIC) and rotation information criterion (RIC). From recent studies[1-2], it has been shown that the consistent AIC (CAIC) [3], CAIC with Fisher information matrix (CAICF) [3] and the information complexity (ICOMP) [4] can be other strong candidates to choose the best model for biological networks. In this study, we extent these recent criteria by inserting non-parametric bootstrap methods[5] since from different studies, it has been observed that the bootstrapping improves the accuracy of the estimates when the observations are limited. Thereby, we analyze the performance of these new approaches in real bench-mark and simulated datasets in terms of accuracy and computational time. The results indicate that the bootstap-based criteria have better accuracies with a slight cost in computational demand.

References:

Bülbül, G. B.; Purutçuoğlu, V., *International Journal of Environmental Science and Technology*., **2019**,
Kaygusuz, M. A.; Purutçuoğlu, V., The Model Selection Methods for Sparse Biological Networks. Chapter in: Lecture Notes on Data Engineering and Communications Technologies, Springer, **2019** (In print).
Bozdogan, H., *Pscychometrica*. **1987**, *52* (*3*), 345-370.
Bozdogan, H.; Haughton, D. M. *Computational Statistics and Data* Analysis. **1998**, *28*, 51-76.
Epskamp, S.; Borsboom, D.; Fried, E. I. *Behavior Research Methods*. **2018**, *50*, 195-212.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In vitro Genotoxic and Antigenotoxic effects of *Nanoliposomal Formulation of Satureja hortensis* Essential Oil Prepared by Dynamic High-Pressure Microfluidization

[a]Münevver Müge Çağal, [a]Gökçe Taner, [b]Gülengül Duman, [c]Ali Yılmaz, [c]Nagihan Nizam, [d]Fikrettin Şahin

[a]*Department of Bioengineering, Bursa Technical University,16310, Bursa, Turkey*

[b]*Faculty of Pharmacy, Yeditepe University, 34755, Istanbul, Turkey*

[c]*Department of Biotechnology, Graduate School of Natural and Applied Sciences, Bursa Technical University,16310, Bursa, Turkey*

[d]*Department of Genetics and Bioengineering, Yeditepe University,34755, Istanbul, Turkey*

munevver.cagal@btu.edu.tr

Satureja hortensis (summer savory) is an annual herb belonging to the family Lamiaceae and used as a traditional folk medicine to treat infectious diseases and disorders. Various studies have suggested that the activity of *Satureja hortensis* may relate to the strong antioxidant properties of its secondary metabolites. To improve its bioavalibility and biological activities we recently developed the nanoliposomal formulation of its essential oil by dynamic high-pressure microfluidization.

Essential oil of *S. hortensis* were prepared into the nanoliposomal drug delivery systems by microfluidization technique to provide the most homogeneous flow to produce the smallest droplet sizes. The essential oil were incorporated into nanoliposomes by ratio (2:1) using high speed homogenizer (at 20.000 rpm) and characterized. Then the essential oil of *S.hortensis* were successfully encapsulated in phospholipid based nanoliposomes. In this study our aim was to evaluate the both genotoxic and antigenotoxic effects of the nanoliposomal formulation of *S. hortensis* essential oil against mitomycin-C (MMC) in human lymphocytes. For the evaluation of genotoxicity and antigenotoxicity cytokinesis-block micronucleus (CBMN) assay in human lymphocytes were used. Human peripheral blood lymphocytes cultures were treated with 4 different concentrations (0.25, 0.5, 1, 2 µg/ml) of nanoliposomal formulation alone to assess genotoxicity and simultanously with 0,2 µg/ml MMC for antigenotoxicity. In all sets of experiments, an untreated negative control, as well as a positive control (MMC) was also run. After preparation of the slides binucleated cells surrounded by well-preserved cytoplasm were scored for the presence of micronucleus.

Micronucleus frequency was shlightly increased at all concentrations when compared with the negative control. This increase was not statistically significant at low concentrations but it has been found to be significant at higher concentration. On the other hand it has been determined in the cultures simultanously treated with MMC and the nanoliposomal formulation of *Satureja hortensis* essential oil that micronucleus frequency was decreased compared to positive control.

Keywords: *Satureja hortensis*, essential oil, nanoliposomal formulation, micronucleus, human lymphocyte culture

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Determination of Antioxidant and Anticancer Properties of Phloroglucinol

Meryem Topal

Gumushane University, Vocational School of Health Services, Gumushane, Turkey

topalmeryem@hotmail.com

Antioxidants neutralize free radicals which attack cells or molecules that reduce the effect.[1] Phloroglucinol is an organic compound. It is used in the synthesis of drugs and explosives.[2] In order to evaluate radical scavenging activity and antioxidant of phloroglucinol, diverse *in vitro* methods such as $DMPD^{\bullet+}$, $DPPH^{\cdot}$, $ABTS^{\bullet+}$ scavenging activity, Cu^{2+} reduction capacity, Fe^{3+}-TPTZ reduction capacity by FRAP method, Fe^{3+} reduction capacity by Fe^{3+}-Fe^{2+} transformation method and Fe^{2+} chelating activities using by bipyridyl reagent.[3] Phloroglucinol was compared with the four standard antioxidant agent such as BHA, trolox, α-tocopherol and BHT were used as the reference antioxidant compounds. For each standard and phloroglucinol, IC_{50} values of $DPPH^{\cdot}$ scavenging activity were calculated; Trolox (7.29 µg/mL), BHA (8.56 µg/mL), α -Tocopherol (8.77 µg/mL), Phloroglucinol (23.10 µg/mL), BHT (28.88 µg/mL). Phloroglucinol strong antioxidant and radical scavenging effects in all methods used.

References:
Topal, F.; Nar, M.; Gocer, H.; Kalin, P.; Kocyigit, U. M.; Gulcin, I.; Alwasel, S.H. J. Enzyme Inhib. Med. Chem. 2016, 31, 674-683.
Demir, Y.; Isık, M.; Beydemir, S.; Gulcin, I. J BiochemMol Toxicol. 2017, e21936.
Gulcin, I.; Topal, F.; Ozturk Sarikaya, S.B.; Bursal, E.; Goren, A.C.; Bilsel, M. Rec. Nat. Prod. 2011, 5, 158-175.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Computer-Aided Exploration of New Lead Compounds as Vascular endothelial growth factor receptor-2 (VEGFR-2) Inhibitors

Mehmet VAROL

Department of Molecular Biology and Genetics, Faculty of Science, Kotekli Campus, Mugla Sitki Kocman University, Mugla TR48000, Turkey.

mehmetvarol@mu.edu.tr

Angiogenesis, the formation of new capillary vessels from the existing vessels is a critical hallmark in cancer progression which facilitates tumor growth by providing oxygen and nutrients, and removing the metabolic wastes, and allows tumor cells for metastasis into other tissues[1]. Vascular endothelial growth factor receptor-2 (VEGFR-2) that is a key element of angiogenesis, considered as an important pharmaceutical target to inhibit angiogenesis and metastasis, and a considerable scientific effort along with a vast financial support has been consumed to discover efficient VEGFR-2 inhibitors[1]. Here, the ligand-based pharmacophore mapping and atom-based 3D-Quantitative structure activity relationship (3D-QSAR) model were carried out based on data set from three confirmatory bioassays available at the PubChem database using Phase program[2]. Six point pharmacophore (DHHRRR) of VEGFR-2 inhibitors was produced from training set of 49 compounds that was determined by considering the diverse structures. The 3D-QSAR model was generated by applying partial least-squares (PLS) algorithm, and chosen as having favorable statistic measures (R^2=0.55, Q^2_{ext}=0.14) for the training set and test set respectively. New lead compounds (428) have been identified from the databases; PubChem (311,268), AfroDb (885), Analyticon (11,247), HIM (663), HIT (802), Indofine (144), NPAC (1,423), NuBBE (588), SPEC (1,489), UEFS (473), IBScreen (84,214), TCM (36,043) and Asinex (286,342) by aiding of the obtained pharmacophore hypothesis. Afterwards, a multistep molecular docking study was performed on the retrieved hits and 11 final compounds that have high docking scores were prioritized, as potential leads against VEGFR-2, which correlated binding mode to experimentally proven compounds and constructive drug-like properties (Figure 1). Consequently, the obtained results provide important information about the structural insights and the binding features of these compounds, which can pioneer the design and development of novel anti-angiogenic agents that target VEGFR-2.

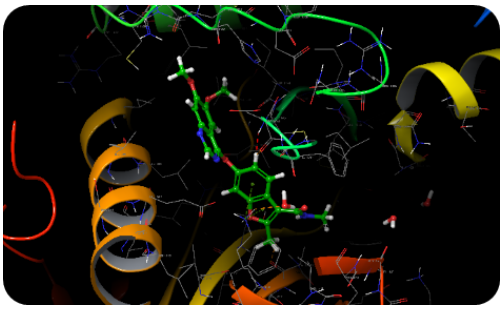


Figure 1. Binding mode of the most active compound (PubChem_44480399).

References:

Varol, M. *Cytotechnology* **2018**, 70, 1565.
Kim, S.; Thiessen, P.A.; Bolton, E.E.; Chen, J.; Fu, G.; Gindulyte, A.; Han, L.; He, J.; He, S.; Shoemaker, B.A.; Wang, J.; Yu, B.; Zhang, J.; Bryant, S.H. *Nucleic Acids Res*. **2016**, 44, D1202.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Neuroprotective and Antioxidant Effects of *Artemisia Absinthium* L.

Mesut Işık[a]

[a]*Department of Pharmacy Services, Vocational School of Health Services, Harran University, 63000, Şanlıurfa, Turkey*
misik@harran.edu.tr

Artemisia absinthium L. (Asteraceae), which is grown in Turkey and many countries has long been used as a traditional herbal medicine for the development of mental function and the treatment of gastric pain and cardiac stimulation in China, Europe and Pakistan[1]. Therefore, we investigated the anticholinergic effec and antioxidant activity of different extracts of the medicinal plant. The antioxidant activity of the extracts in the study was investigated using total phenolics content, radical scavenging capacity by DPPH and ABTS and metal reduction capacity as CUPRAC and FRAP methods. Furthermore, the effect of the water and methanol extracts of Artemisia absinthium L on acetylcholinesterase (AChE) that has been hydrolyzed the neurotransmitter acetylcholine and on paraoxonase 1 (PON1), which plays an important role in the prevention of lipid peroxidation by regulating HDL-LDL levels was investigated. When DPPH and ABTS radical removal activities were analyzed, *Artemisia absinthium* L. was found to be 24.87, 28.72% in water extract and 18.77, 9.06% in methanol extract respectively, for 1000 µg / mL concentration. The water and methanol fraction of *Artemisia absinthium* L. was exhibited in vitro the strongest inhibition on AChE with IC50 values of 0,067 and 0,164 mg/ml, respectively, while its had no effect on PON1. The results showed that different fractions has moderate metal reducing capacity, free radical scavenging and high anticholinesterase effect. In conclusion, it is thought that *Artemisia absinthium* L can be used as an alternative natural medicine instead of synthetic drugs used in the treatment of Alzheimer's patients because of its antioxidant and neuroprotective effect.

Keywords: Acetylcholinesterase, radical scavenging, neuroprotective effect, *Artemisia absinthium* L

References (optional):

Bora K.S., Sharma, A. Neuroprotective effect of Artemisia absinthium L. on focal ischemia and reperfusion-induced cerebral injury. *Journal of ethnopharmacology*, 2010, 129(3), 403-409.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Ultrasound-assisted Synthesis of Novel Potential Biological Active 1,4-Dihydropyridines

Mehtap Özgür[a] and Duygu Bayramoğlu[a]

[a] *Ankara University, Faculty of Science, Department of Chemistry, 06100, Ankara, Turkey*

mehtapyakut@gmail.com

Heterocycles containing 1-4-dihydropyridines (1,4-DHP) are a class of compounds, which are recognized in the field of medicine and organic chemistry due to their biological activities such as antitumor, analgesic, hypnotic and anti-inflammatory.[1] Furthermore, some important prescribed drugs such as *amplodipine* and *nicardipine* (**Fig. 1**) are known as a major Ca^{+2} channels blockers, that are used for treated of hypertension.[2]

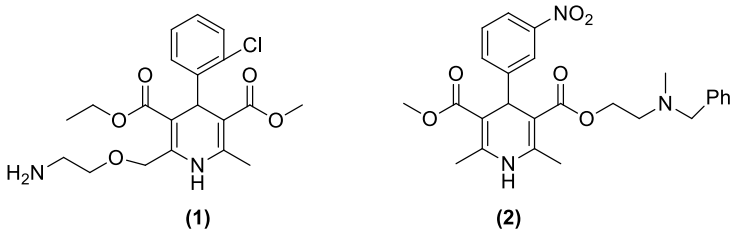


Figure 1. Structure of *amplodipine* (1*)* and *nicardipine* (2)

Herein, we report an efficient, simple and green procedure for the synthesis of new dihydropyridine derivatives (**5**) in an aqueous solvent via a one-pot multicomponent reaction of ethyl acetoacetate (**1**), aromatic aldehydes (**2a–e**) and heteroaromatic aldehydes (**3a-b**), ammonium acetate (**4**), without catalyst using ultrasonic irradiation (**Fig. 2**).

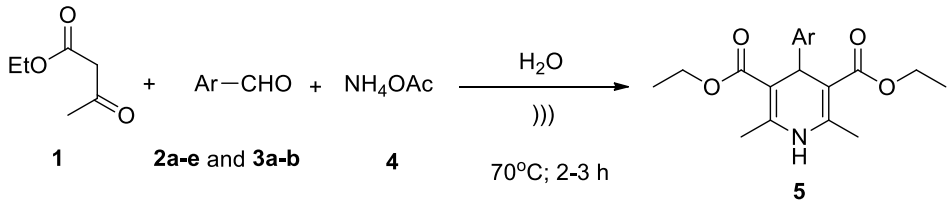


Figure 2. Synthesis of novel dihydropyridine derivatives

References:

Tayebeh Momeni, J. Molec. Struc. **2020**, 1199, 127011.
Jamal Davarpanah, Journal of Molecular Structure. **2019**, 1177, 525.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Development of Combined HPLC-DAD-FRAP-Spectrophotometric Carbonic Anhydrase Inhibitor (CAI) Method and Application to Some Plant Extracts

Semra Alkan Türkuçar[a], Murat Küçük[b], Ayça Aktaş Karaçelik[c], Melek Kaya[b], Berna Senem Kefeli[d]

[a]*Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Nutrition and Dietetics Department, Alanya, Antalya, Türkiye*
[b]*Karadeniz Technical University, Faculty of Sciences, Department of Chemistry, Trabzon, Türkiye*
[c]*Giresun University, Espiye Vocational School, Department of Food Processing, Giresun, Türkiye*
[d]*Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Türkiye*
semra.turkucar@alanya.edu.tr

Carbonic anhydrase (CA, EC 4.2.1.1) is a highly dynamic research area. Modulators of most CA isoenzymes are used for the treatment of diseases such as edema, glaucoma, obesity, cancer, epilepsy and osteoporosis with potential inhibition or activation effects.[1] On-line HPLC applications offer significant advantages in the determination of bioactive components from natural products. Recently, on-line post-column bioactivity determination methods have been widely used.[2] The previously developed on-line HPLC-FRAP antioxidant method[3] was used together with CAI detection method, and combined HPLC-DAD-FRAP-Spectrophotometric CAI method was applied to sumac and dianthus plant extracts. The advantages of the method have been demonstrated. The new method can be used to detect compounds with high CAI activity, with IC_{50} values below 50 µM, and high clinical value. Therefore, the on-line and combined methods developed can be used as an important tool in the study of high CAI activity compounds with potential for clinical applications.

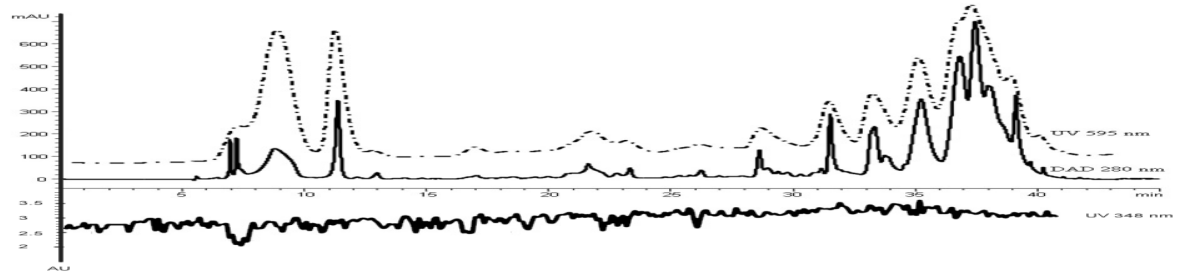


Figure 1. Chromatograms at 280 nm (DAD) for compound detection and at 595 nm (UV) for FRAP activity determination and the graph of absorbance at 348 nm showing CA esterase modulation.

*This study was supported by 2211-C Priority Areas Domestic PhD Scholarship Program of TUBITAK-BIDEB.

References:

Supuran C. T.; Scozzafava A. *Exp. Opin. Ther. Patents*. **2002,** 12, 217-242.

Shu-Yun S.; Hong-Hao Z.; Yu-Ping Z.; Xin-Yu J., vd. *Trends in Analytical Chemistry*. **2009,** 28, 865-877.

Arslan Burnaz N.; Küçük M.; Akar Z. *Journal of Chromatography B.* **2017,** 1052, 66-72.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Talaromyces marneffei Mp1 protein, a novel virulence factor, carries two arachidonic acid-binding domains to suppress inflammatory responses in hosts

Kong-Hung Sze[a,b,c], Wai-Hei Lam[d], Hongmin Zhang[d,e], Man-Kit Tse[a,b,c], Pok-Man Lai[a,b,c], Ting Zhou[a,b,c], Timothy Tin-Mong Yung[a,b,c], Patrick C.Y. Woo[a,b,c], Susanna K.P. Lau[a,b,c], Richard Yi-Tsun Kao[a,b,c], Kwok-Yung Yuen[a,b,c], Quan Hao[d]

[a]State Key Laboratory of Emerging Infectious Diseases ;[b]Department of Microbiology , Li Ka Shing Faculty of Medicine; [c]Carol Yu Centre for Infecti; [d]School of Biomedical Sciences, The University of Hong Kong, Hong Kong Special Administrative Region, Chi; [e]Department of Biology and Shenzhen Key Laboratory of Cell Microenvironment,Southern University of Science and Technology, Shenzhen 518055, China.

khsze@hku.hk

Talaromyces marneffei (T. marneffei) infection causes talaromycosis (previously known as penicilliosis), the second most-deadly opportunistic systematic mycosis in immuno-compromised patients. Different virulence mechanisms in *T. marneffei* had been proposed and investigated. In the sera of patients with talaromycosis, Mp1 protein (Mp1p), a secretory galactomannoprotein antigen encoding two tandem ligand-binding domains (Mp1p-LBD1 and Mp1p-LBD2), was found to be abundant. We recently showed that Mp1p is a novel virulence factor of *T. marneffei.* Yet the mechanism governing its virulence was unknown. We have performed pull-down of Mp1p protein against the *T. marneffe*i infected cell lysates of J774 macrophage cells. LC-MS characterization of the pull-down extract indicated that the Mp1p-LBD2 has high affinity for a key human proinflammatory lipid mediator, arachidonic acid (AA). The first line defense of our body against microbial infection is achieved through inflammatory response, which is a complex but highly coordinated series of events tightly controlled by a number of mediators, many of which are derived from AA or its metabolites. Therefore, we have performed structure, function and binding investigations of Mp1p against AA by combining LC-MS, structural biology and cell biology approaches. The crystal structure of Mp1p-LBD1-LBD2 has also been solved, showing that both LBDs each can bind up to 2 AA molecules and they are likely to function independently with a flexible linker in between. Finally we showed in cell-based LC-MS lipidomics that the AA capturing property of Mp1p is functionally relevant because *T. marneffe*i is able to reduce the availability of cellular AA and decrease the production of downstream eicosanoids. Adding together, these results support that Mp1p is an important virulence factor for intracellular survival of *T. marneffei* through trapping of pro-inflammatory mediators. This work has provided important insight into the host defense evasion and pathogensis of T. marneffei, which may extend to other fungi or pathogenic bacteria. Such knowledge may help to develop better chemotherapautic intervention of fungal diseases in future.

Acknowledgements: Research Grant Council Fund of Hong Kong (GRF17124717).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In silico structure-based screening of large ligand library against virulence factors of drug resistant pathogens

Kong-Hung Sze[a,b,c], Timothy Tin-Mong Yung[a,b,c], Pok-Man Lai[a,b,c], Ting Zhou[a,b,c], Man-Kit Tse[a,b,c], Richard Yi-Tsun Kao[a,b,c], Kwok-Yung Yuen[a,b,c]

[a] State Key Laboratory of Emerging Infectious Diseases ;[b] Department of Microbiology , Li Ka Shing Faculty of Medicine;; [c] Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative Region, China,

khsze@hku.hk

The emergence of drug-resistant microbes is an alarming threat to the global population. There is an urgent need of effective drugs against these pathogens. Among these, *Staphylococcus aureus* (SA) and *Mycobacterium tuberculosis* (MTB) are especially problematic to human. The global spread of MRSA and multi-drug resistant tuberculosis (MDR-TB) are of great concern because they are quickly acquiring resistance to all clinical antibacterial agents. A novel approach of drug development is to target virulence factors, which can potentially prevent drug-resistance from building up. Early secretory proteins SA EsxA and MTB ESAT6/CFP10 are known to be virulence factors which plays major role in the pathogenesis of these bacteria. Therefore they are promising drug targets. An *in silico* platform has been setup to perfrom structure-based screening of a large compound library containing 6.8 million lead-like and bioactive ligands against these virulence factors. Clustering analysis on the docking results led to the prediction of important binding sites on EsxA and ESAT6/CFP10. Out of the top 100 docking score compounds, 5 and 4 hit compounds targeting EsxA and ESAT6/CFP10, respectively, were validated by secondary screening using nuclear magnetic resonance binding assays. The effectiveness of these hit compounds were futher evulated by measuring their minimal inhibitory concentrations by broth microdilution assay and intracellular sruvival assay in J774 macrophage cells. The present study using the *in silico* structure-based screening platform has laid the foundation for drug development targeting EsxA and ESAT6/CFP10. The resulting confirmed hit compounds will be useful leads to develop therapeutic drugs to combat MRSA and MDR-TB.

Acknowledgements: Research Grant Council of Hong Kong; Health and Medical Research Fund of Hong Kong(13120862, 14131142, HKM-15-M05) and the Research Grant Council Fund of Hong Kong (GRF17124717).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

A POLYOXY GROUP BRANCHED DIAZO DYE MODIFIED ELECTROCHEMICAL SENSOR FOR EPINEPHRINE DETECTION

Tuğba ÖREN VAROL, Okan AVCI, Benay PERK, Yudum TEPELİ BÜYÜKSÜNETÇİ, Özgül HAKLI, Ülkü ANIK

Muğla Sıtkı Koçman University, Faculty of Science, Chemistry Department, Kötekli, Muğla, 48000, Turkey

tugbaoren@mu.edu.tr

Epinephrine, also known as adrenaline, is a catecholamine neurotransmitter with pharmacological effects on mammalian central nervous system and glycogen metabolism. Epinephrine concentration in biological fluids may affect heart rate, plasma lactate level, lipolysis and glycogenolysis. Besides, epinephrine level in biological samples has been evaluated as a biomarker for the diagnosis of Parkinson's disease, Huntington's disease and schizophrenia.[1,2] Thus, sensitive and practical detection of epinephrine in biological samples as well as in pharmaceutical formulations are demanded.

In the present work, the main idea was to develop an efficient modified electrochemical platform with a novel polyoxy group branched diazo dye for the epinephrine detection. Within this purpose, effects of diazo dye amount, pH and scan rate on epinephrine peak current values were investigated. After the optimization of experimental parameters, analytical characteristics were examined. As a result, a broad linear range was obtained between 0.1-75 µM with the limit of detection value of 0.013 µM (n=3). Interference study was conducted in the presence of uric acid and the developed sensor was tested for the epinephrine detection in adrenaline injection samples. The obtained results revealed that the polyoxy group branched diazo dye modified sensor can be alternatively used for the practical and sensitive epinephrine detection in clinical applications.

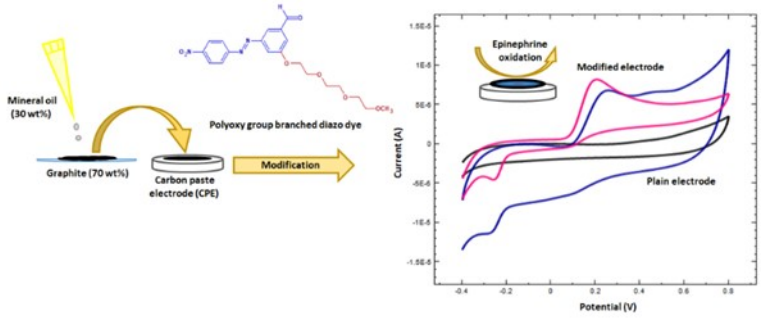


Figure 1. Preparation of the polyoxy group branched diazo dye modified electrode

References:

Tomé, L. I. N.; Brett, C. M. A. *Electroanalysis* **2019**, *31*, 704.
Anithaa, A. C.; Asokan, K.; Sekar, C. *Electrochim. Acta* **2017**, *237*, 44.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Development and Characterizatıon of Nanocarrier System for Inhaler Chemotherapy

Güliz Ak
Ege University Faculty of Science Biochemistry Department, Izmir, Turkey
guliz.ak@gmail.com

Lung cancer is the most frequently occured cancer type and causes 23% of total cancer related death in worlwide. Pulmonary drug delivery for lung cancer therapy provides local enhanced drug concentration, descreased required drug dose and eventually decreased systemic side effects. Doxorubicin is widely used for treatment of many cancer types such as breast, ovarian and lung cancer. The objective of this study was development of inhalable doxorubicin carrying nanoparticular system. Alginate and chitosan which have mucoadhesive properties were used as biopolymers for nanoparticle synthesis. Alginate/chitosan nanoparticles were prepared and bound with doxorubicin via PEGdiacid spacer with amide bond. FTIR, zetasizer and SEM analyses were perfomed in all steps and then in vitro drug release tests were carried out. It was found that doxorubicin carrying nanoparticles had 0.44 mg drug in per mg nanoparticle, hydrodynamic size as 217.23 nm±15.00 and zeta potential as -25.17 mV±2.67. More drug release rate was determined in acidic medium than that of physiological medium. According to the data, it is thought that doxorubicin bound alginate/chitosan nanoparticles could have potential for further studies in terms of treatment of lung cancer through inhaler route.

Acknowledgement: I would like to thank Ege University Scientific Research Project Office (Grand no: 17 FEN 014) for the financial support.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Non-Gaussian Model Construction of Biological Networks via Copulas

Hajar Farnoudkia[a], Vilda Purutçuoğlu[a]

[a] *Department of Statistics, Middle East Technical University, Ankara, Turkey*

vpurutcu@metu.edu.tr

The graphical representation of biological networks is one of the convenient ways for researchers due to its simplicity in the description of the biological activation between genes. The Gaussian graphical model (GGM) [1-2] is one of the common approaches in this field. Although, this method is successful for small and moderate systems when the states of genes are multivariate normally distributed, it has limitations due to dimensional and distributional constraints. In order to unravel these problems, several alternative solutions have been suggested recently. For instance, GGM is converted to the Gaussian copula graphical model (GCGM) by partitioning the system via the Gaussian copula and conducting the inference via distinct Bayesian algorithms. This method solves the problems of high dimension, whereas, it is still dependent on the normality assumption[3]. Hereby, the D- and C- vine copula graphical models have been proposed to deal with non-Gaussian datasets[4] . In these new models, the inference is conducted by the penalized likelihood approach. From the analyses via toy sets it has been shown that they can be a strong candidate to present complex biological systems. In this study, we extend these analyses by evaluating the performances of both vine copula models in different simulated data and assess their accuracies via various accuracy measures comprehensively. The results indicate that the vine copulas can more realistically describe the biological networks with significant computational gain with respect to the GCGM-based models.

References:

Whittaker, J. *Graphical Models in Applied Multivariate Statistics.* **1990**, John Wiley and Sons, New York.

Purutçuoğlu, V.; Farnoudkia, H. *Acta Physica Polonica A*. **2017**, *132*, 1112-1117.

Farnoudkia, F.; Purutçuoğlu, V. *Scientia Iranica*. **2019**, *26* (*4*), 2495-2505.

Farnoudkia, F.; Purutçuoğlu, V. *Proceeding of 5th International Conference on Engineering Sciences.* **2019** (In print).

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Antibacterial vs. Anti-Biofilm Effect of Azithromycin on *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients in Iran

Somayeh Moazami Goudarzi [a], Ahya Abdi Ali [a], Mohammadreza Modaresi [b], Tooba Ghazanfari [c]

[a] *Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran*
[b] *Pediatric Pulmonary Disease and Sleep Medicine Research Center, Pediatric Center of Excellence, Children's Medical Center, Tehran, Iran*
[c] *Immunoregulation Research Center, Shahed University, Tehran, Iran*

abdialya@alzahra.ac.ir

Introduction: Cystic fibrosis (CF) is an autosomal recessive disease characterized by respiratory, gastrointestinal, and reproductive tract problems. Most involvement, morbidity and mortality in patients with CF result from *Pseudomonas aeruginosa* chronic pulmonary infection which frequently leads to pulmonary exacerbations. In CF, It has been shown that macrolides such as azithromycin (AZM) have beneficial effects like an anti-inflammatory, antivirulence and anti-biofilm activity.In the present study, we evaluated the ability of biofilm formation among *P. aeruginosa* isolates from Iranian CF patient as well as comparison of antibacterial and anti-biofilm effect of the AZM for the first time in Iran.

Materials and methods: Antibacterial effect (MIC) of azithromycin on 21 *P. aeruginosa* isolates from Iranian CF patients were studied. Moreover, we investigated biofilm formation ability as well as anti-biofilm effect of azithromycin on biofilm producer *P. aeruginosa* isolates.

Results: The results showed high level of MIC (≥512 µg/ml); most isolates (70%) were biofilm-producing strain amongst which 20% were strong producers. The results of the anti-biofilm effect of azithromycin showed that sub-MIC concentration (≥64 µg/ml) can inhibit biofilm production.

Conclusion: Although azithromycin showed high level of MIC and weak antibacterial effect on *P. aeruginosa* isolates from CF but good anti-biofilm effect in low dose was observed which may affect dangerous chronic pulmonary infection by *P. aeruginosa*. It is due to the antivirulence activity of AZM that may result in direct and/or indirect repression of specific subsets of genes involved in virulence, quorum sensing, biofilm formation, and intrinsic antibiotic resistance.

Keywords: *Cystic Fibrosis, P. aeruginosa*, Azithromycin, Antibacterial, Anti-biofilm

2EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The evaluation of chloroform extract obtained from the roots and aerial parts of *Ferulago cassia* Boiss. (Apiaceae) on lipopolysaccharide-induced human umbilical vein endothelial cell injury

[1]Zekai HALICI, [2]Zerrin KUTLU

hzekai@gmail.com, kutluzerrin@atauni.edu.tr

[1]Faculty of Medicine, Pharmacology Department, Ataturk University, 25240, Erzurum, Turkey.
[2]Faculty of Pharmacy, Biochemistry Department, Ataturk University, 25240, Erzurum, Turkey.

Aim. *Ferulago* species have been utilised as sedative, flavor, tonic, peptic, aphrodisiac, antibronchitis, immunostimulant, carminative, and for the therapy of cancers and skin disorders in traditional medicines. The presented study reports antiinflammatory capacities of aerial parts and roots chloroform extracts of *F. cassia.*

Material. The used extracts were obtained according to the method of Karakaya et al., 2019 (1). Human umbilical cord vein endothelial cell line (HUVEC) was used. In the lipopolysaccharide (LPS) induced endothelial cell injury, groups were determined as control (C), LPS (L), chloroform extract of roots (RC), LPS + RC, chloroform extract of ariel parts (APC), LPS+ APC. HUVEC cells (5×10^3 cells/mL) were seeded in 96-well plates and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Firstly, inhibition concentration 50 (IC_{50}) values of extracts (RC and APC) were determined. We found that IC_{50} values of chloroform extract of roots (0.5 mg/mL) and chloroform extract of ariel parts (0.5 mg/mL). Then the cells were treated with these extracts at 2 µL concentration. After 1 hour, 1 µg/mL LPS was added in each wells. The cells were further incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 hour, 48 hour and 72 hour. 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to the cell culture. 100 µL of MTT solution after 4 hours of incubation was added. The optical density of formazan solution was measured with a microplate reader at 570 nm.

Result and Discussion. In the LPS group, it was determined that cell proliferation were decreased at the 24th, 48th and mostly at 72th hours. Cell inhibition were found at the 24th (%50.64), 48th (%68.18) and 72th (%81.23). There were no significant differences in RC, and APC groups compared to the control group.

References

S. Karakaya, M. Koca, O. Sytar, B. Dursunoglu, H. Ozbek, H. Duman, Z. Guvenalp, C.S. Kılıc. Antioxidant and anticholinesterase potential of Ferulago cassia with farther bio-guided isolation of active coumarin constituents. South African Journal of Botany. Volume 121, March 2019, Pages 536-542.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The evaluation of aqueous extract obtained from the roots and aerial parts of *Ferulago cassia* Boiss. (Apiaceae) on lipopolysaccharide-induced human umbilical vein endothelial cell injury

Zerrin KUTLU
kutluzerrin@atauni.edu.tr
Faculty of Pharmacy, Biochemistry Department, Ataturk University, 25240, Erzurum, Turkey.

Aim. *Ferulago* species have been utilised as aphrodisiac, sedative, flavor, tonic, digestive, antibronchitis, immunostimulant, antiflatulent, and for the healing of cancers and skin disorders in traditional medicines. The presented study reports antiinflammatory capacities of aerial parts and roots aqueous extracts of *F. cassia.*

Material. The utilized extracts were gained according to the method of Karakaya et al., 2019. Human umbilical cord vein endothelial cell line (HUVEC) was used. In the lipopolysaccharide (LPS) induced endothelial cell injury, groups were determined as control (C), LPS (L), water extract of roots (RW), LPS + RC, water extract of ariel parts (APW), LPS+ APW. HUVEC cells (5×10^3 cells/mL) were seeded in 96-well plates and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. Firstly, inhibition concentration 50 (IC_{50}) values of extracts (RW and APW) were determined. We found that IC_{50} values of water extract of roots (0.5 mg/mL) and water extract of ariel parts (0.5 mg/mL). Then the cells were treated with the 2 µL these extracts. After 1 hour, 1 µg/mL LPS was added in each wells. The cells were further incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 24 hour, 48 hour and 72 hour. 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to the cell culture. 100 µL of MTT solution after 4 hours of incubation was added. The optical density of formazan solution was measured with a microplate reader at 570 nm.

Result and Discussion. In the LPS group, it was determined that cell proliferation were decreased at the 24th, 48th and mostly at 72th hours. Cell inhibition were found at the 24th (%52.58), 48th (%63.28) and 72th (%85.03). There aren't significantly differences in RW, and APW groups compared to the control group.

References

S. Karakaya, M. Koca, O. Sytar, B. Dursunoglu, H. Ozbek, H. Duman, Z. Guvenalp, C.S. Kılıc. Antioxidant and anticholinesterase potential of Ferulago cassia with farther bio-guided isolation of active coumarin constituents. South African Journal of Botany. Volume 121, March 2019, Pages 536-542.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Synthesis and characterization of stimuli-responsive polymers

Tunca Karasu[a], Fatma Zehra Erkoç[b], Canan Armutcu[c], Bora Garipcan[b], Lokman Uzun[a], Mehmet Emin Çorman[a]

[a]*Sinop University, Faculty of Sciences and Arts, Department of Chemistry, Sinop, Turkey*
[b]*Bogazici University, Institute of Biomedical Engineering, Istanbul, Turkey*
[c]*Hacettepe University, Department of Chemistry, Ankara, Turkey*

(lokman@hacettepe.edu.tr)

Over the past 25 years, "stimuli-responsive" have been proposed for many interesting biomedical uses especially in the areas of drug delivery, regenerative medicine and cell culture surfaces due to their rapid respond to small changes in environmental stimuli.[1] Stimuli-responsive polymers can be developed as artificial extracellular matrixes (ECM) that might mimic the natural tissue microenvironment with the possibility of manipulating their physical or chemical properties by bioactive molecules.[2] Therefore, developing a biomaterial to mimic tissue microenvironment in terms of topographic, biochemical and mechanical strength are benefical for cultivation of cells.

In this study cell sheets were developed by mimicking cell microenvironment in terms of physical, biochemical and mechanical strength for cell culture and investigate cell viability and profileration on new generation regenerative artificial cell sheets. Stimuli-responsive polymers were synthesized by incorporating N-methacryolyl-L-glutamic acid as functional monomer into the polymer backbone that yield the polymer response. Characterizations of polymers were studied using Fourier transform infrared, thermogravimetric analysis and scanning electron microscopy. Additionally, Stimuli-responsive polymers were improved with surface modification techniques by incorporation of collagen/hyaluronic acid.

*This study was supported by The Scientific and Technological Research Council of Turkey with a grant number as TUBITAK-KBAG-117Z166

References:

Annabi,N., Tamayol, A., Uquillas, J. A., Akbari, M., Bertassoni, L. E., Cha, C., Unal, G.C., Dokmeci, M. R., Peppas N. A., Khademhosseini, A. **2014**. *Advanced Materials*, 26, 85–123.

Chen, Y.S., Tsou, P.C., Lo, J.M., Tsai, H.C. Wang, Y.Z., Hsiue, G.H. **2013**. *Biomaterials*, 34, 7328-7334.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

DeCoBac as a solution of microbiological soil remediation

Martin Wagner [1], Arzu Javadova [2]

MicroPro GmbH, Magdeburger Str. 26 b, 39245 Gommern, Germany
[1]-General Manager; [2]-Business Development Manager,
arzujavadova@yahoo.com

One approach to clean up organic contaminations, e.g. oil or fuel spills from soil, is the microbiological remediation process. Several contaminations can easily and cost-effective degraded by naturally occurring (autochthonic) or additionally introduced (allochthon) micro-organisms. The soil-innate micro-flora can often be activated by addition of missing nutrients or oxygen. With these simple measures an increased microbial activity can result in an effective degradation process.

DeCoBac is a stable mixture of bacteria developed by German MicroPro company, specifically selected and adapted to degrade a wide range of hydrocarbons under aerobic conditions. In case the autochthonic (on-site) soil micro flora does not degrade hydrocarbon contaminations, the degradation potential can significantly be increased by addition of active bacteria culture (e.g. in soil treatment facilities). Extensive laboratory tests and field applications in various soil treatment facilities have confirmed a successful application under practical conditions. DeCoBac is a ready-to-use liquid formulation of highly active bacterial cultures that has been specially developed for soil remediation. It consists of acomplex mixture of selected bacterial strains that have been adapted to the specific remediation tasks in order to target the mineral oil hydrocarbons present in the soil. Features and benefits of DeCoBac are: Fast degradation of hydrocarbons; Not corrosive; Non-pathogenic; Safe to handle; Can be used immediately in the delivered form; Especially effective in combination with selected surfactants. Bacteria in DeCoBac needs suitable conditions for the maximum performance : pH range: from 6.5 to 8.0 (best value near pH 7.0); 50 % of maximum water holding capacity of soil; Temperature influences the activity of the working solution; Process increases with rising temperatures up to 50 ° C;Below 5 ° C no activity can be expected. This innovative method of a microbiological soil remediation successfully deals with the removal of pollution or contaminants from environmental media such as soil, groundwater, sediment, or surface water.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Protection of hepatic tissue via glutathione system during the acute inflammation

Berna HUKKAMLI[a], Harun BUDAK[a]

[a]*Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum,Turkey*

bernahukkamli1@gmail.com

Inflammation is a reaction of the immune system to pathogens, damaged tissues, and toxic agents. The inflammation process is divided into acute and chronic, depending on the duration and amount of severity.[1] Acute inflammation, the first response to tissue damage and infectious agents, continues for a long time, the inflammatory process gains a new character that turns into chronic inflammation.[1] Chronic inflammation induces the production of reactive oxygen species (ROS) that lead to oxidative stress, provoking wide variety of diseases including cardiovascular diseases, cancer, diabetes, obesity, arthritis, Alzheimer, pulmonary diseases, and autoimmune diseases.[1] The antioxidant system neutralizes the ROS to protect the cell against the oxidative stress. Since the enzymatic antioxidant system containing the thioredoxin and glutathione system uses nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor, there is a connection between them through NADPH.[2] In our previous study, we demonstrated how the hepatic thioredoxin system in mouse liver was affected in the case of acute inflammation at the protein and gene levels . The question now is how the glutathione system will act at the protein level during acute inflammation.[3] Gene expression levels of the *Il-1* and *Il-6* cytokines, which is a marker for the inflammation[4], were examined by Real-Time PCR to represent inflammation model in male mice liver injected intraperitoneally with lipopolysaccharide (LPS). Any significant change in the reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio, which is a marker of oxidative stress[5], was measured spectrophotometrically. After the model was established, the enzyme activity of the glutathione mechanism including glutathione reductase (GR) and glutathione peroxidase (GPx) were determined spectrophotometrically. Since the increase in the *Txnip* expression is a precursor for the apoptosis pathway to be active, its expression was also measured by Real-Time PCR. The results showed that while the expression of *Il-1*, *Il-6*, and *Txnip* was elevated, the GSH/GSSG ratio was decreased during acute inflammation. Furthermore, GPx and GR activities were decreased. According to the results, it might be suggested that the glutathione system decreases its own activity in the case of acute inflammation, contributing to the cell's orientation to apoptosis, thereby preventing chronic inflammation that leads to many diseases and especially the cancer.

References

Franceshi, C.; Campisi, J. *The Journals of Gerontolog.* **2014**, 69,1:S4-9.

Metere, A.; Frezotti, F.; Graves, C. E.; Vergine, M.; De Luca, A.; Pietraforte, D.; Giacomelli, L. *Cancer Cell International* **2018**, 18(1): 7.

Hukkamlı, B.; Budak, H. 1 st Eurasia Biochemical Approaches Techonologies (EBAT), **2018**, S-138.

Tschopp, J.; Schroder, K. *Nature Reviews Immunology*. **2010**, 10(3): 210.

Budak, H.; Kocpinar, E. F.; Gonul, N.; Ceylan, H.; Erol, H. S.; Erdogan, O. *Comp Biochem Physiol C Toxicol Pharmacol.* **2014**, 166:44-50.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Antimicrobial Properties of Loganic Acid and Its Effects on Probiotic Bacteria *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*

Hasan Ufuk Çelebioğlu[1], Busenur Çelebi[1], Recep Taş[1], Hüseyin Akşit[2]

[1]*Bartın University, Faculty of Science, Department of Biotechnology*

[2]*Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Analytical Chemistry*

busecelebi5974@gmail.com

Probiotics are defined as the microorganisms, when administered adequetly, that confer health benefits to the host. The most studied and known probiotics are *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*. These bacteria are used as "starters" in various foods or for different formulations, as well as food supplements.

Loganic acid is an iridoid found in various plants. It is especially abundant in Cranberry (*Cornus mas* L.). The anti-inflammatory and protective effects of loganic acid have been proven, but various biological activities have not been studied. The aim of this study is to investigate the antimicrobial effects of loganic acid, as well as effects on probiotic bacteria *L. acidophilus* LA-5 and *L. rhamnosus* GG. For this, its antimicrobial effects against *Escherichia coli* and *Staphylococcus aureus* were investigated by micro-dilution technique and minimum inhibitor concentrations (MIC) were calculated. Furthermore, its effects on surface hydrophobicity and auto-aggregation properties of probiotic bacteria were determined using different concentrations of loganic acid.

In this study, the MICs of the loganic acid were calculated as 110 µg/mL for *E. coli* and 106 µg/mL for *S. aureus*. Furthermore, loganic acid decreased the surface hydrophobicity of *L. acidophilus* LA-5, but did not show a statistically significant effect on the surface hydrophobicity of *L. rhamnosus* GG. >Furthermore, loganic acid showed a general decrease in auto-aggregation.

In this study, antimicrobial activity of loganic acid, whose biological activity has not been studied very well before, has been shown, as well as its probable interactions with probiotic bacteria have been studied. It is planned to study these interactions with more detailed studies.

Acknowledgements: The authors thank to Chr. Hansen, Turkey for probiotic strains.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Interactions of Probiotic Bacteria *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* with Ipolamiid, an Iridoid Glycoside

Hasan Ufuk Çelebioğlu[1], Ebru Köroğlu[1], Recep Taş[1], Hüseyin AKŞİT[2]

[1] *Bartın University, Faculty of Science, Department of Biotechnology*

[2]*Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Analytical Chemistry*

ebru1nisann@gmail.com

Iridoid glycosides are 2-cyclopentanoid derivatives of terpene origin that occur in the leaves, fruits, seeds, bark, roots of plants and are naturally occurring in the dicotyl plant family. They protect plants from biotic attacks and abiotic effects, and are also medically important because they are used in the treatment of many diseases. It has anti-microbial, anti-tumor, anti-cardiac and anti-inflammatory effects on human health. Ipolamiid, one of the iridoid glycosides, is naturally found in many plants and has little work in the literature.

The beneficial microorganisms in the body and the compounds of plant origin interact in the human digestive tract and can interact each other. Probiotics are food ingredients that has many health benefits by improving microbial balance of the intestine. Among the most known and most studied probiotics are *Lactobacillus acidophilus* and *Lactobacillus rhamnosus*.

In this study, the effects of ipolamiide on probiotic bacteria *Lactobacillus rhamnosus* GG (GG) and *Lactobacillus acidophilus* LA-5 (LA-5) were investigated. For this purpose, ipolamiide were added to the media of probiotics in different concentrations and their effects on bacterial growth kinetics, bacterial surface hydrophobicity (Microbial Adhesion to Solvents - MATS Test) and bacterial aggregation (Auto-Aggregation Test) were studied.

In this study, ipolamiide did not show any important change in surface hydrophobicity of probiotic bacteria. Dose-dependent increases in auto-aggregation properties of the LA-5 and GG were observed. However, further studies will give insight into other possible biological activities of ipolamiide.

Key words: Aggregation, Iridoid Glycosides, MATS Test, Probiotics

Acknowledgements: The authors thank to Chr. Hansen, Turkey for probiotic strains.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Circadian Gene Interactions: A Circular Approach

Elif Doğan Dar[a], Vilda Purutçuoğlu[a]

[a] *Department of Statistics, Middle East Technical University, Ankara, Turkey*

elif.dar@metu.edu.tr

Circular data contains directional information which is inherently different from linear data. It can represent angles or a recurrent phenomenon which repeats in days, years or seasons. Although there are many sources where circular theory has been examined, complex models involving circular data have not been studied extensively yet. In this work, we are specifically interested in interactions among circadian genes, whose expression levels repeat itself in a daily manner. Recent studies have focused on this problem without taking into account the circular nature of the problem. Here, we build a network model with circadian genes and reveal the interactions among them by using a circular statistical model instead of usual statistical theory.

We extract microarray data which includes circadian genes from public databases such as Gene Expression Omnibus (GEO) from NCBI or ArrayExpress from EBI. Microarray data are linear, although expressions of circadian genes are recurrent in a daily manner, and hence circular. Therefore, we have to transform this linear data to circular data. There are multiple methods suggested in the literature to achieve this. One of them is to apply sine curves to fit the data which are acquired every 2-3 hours for 2-3 days. Period will be 24 hours and amplitude and phase will be estimated from data.

We regress each gene on other genes by applying multiple circular regression model by Kim and SenGupta[1], and using least circular mean-squared estimation (CMSE) for coefficients. These estimators are asymptotically normally distributed. Therefore, we use z-statistics to test whether corresponding coefficients are 0 or not in a step-wise variable selection manner. Since this procedure does not dictate coefficients being statistically significant from the perspective of both genes, we apply an “and” rule to decide which interactions are present. A gene interaction is assumed to exist if both coefficients are statistically significant. With this work, we validate known gene interactions and also suggest unknown candidate interactions for further investigation. This way, earlier unnoticed interactions can be used in further system biology or drug research after experimental approval of the claims.

References:

Kim, S.; SenGupta, A. Multivariate-multiple circular regression *J. Stat. Comput. Simul.* **2017**, *87:7, 1277-1291.*

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The comparison of TXNIP-mediated response of hepatic and renal glutathione systems during iron overload

[a]Feyza Sönmez, [a]Harun Budak

[a]*Department of Molecular Biology and Genetics, Faculty of Science, Atatürk University, Erzurum, Turkey*

fyzsnmz14@gmail.com

Iron is used as a cofactor in basic biochemical pathways such as energy metabolism, DNA synthesis, and oxygen transport in almost all cells. However, the presence of excess iron in the cell catalyzes the formation of iron/hydrogen peroxide (Fe/H_2O_2) bond. This is called the Fenton reaction and allows the formation of highly reactive molecules such as hydroxyl radicals ($^{\bullet}OH$) and hydroxide anions (OH^-).[1,2] These form of oxygen are known as reactive oxygen species (ROS) that are essential for many cellular processes as signal transduction molecules. But, increasing the amount of ROS in the cell causes oxidative stress that damages macromolecular structures including nucleic acid, lipid, protein leading to the neurodegeneration, cancer, and diabetes.[3] Enzymatic antioxidant systems including the thioredoxin and glutathione antioxidant system neutralize the ROS to protect the cell against the damage of oxidative stress by using the nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Thus, there is a link between them through NADPH.[4] In our previous study, we reported that how the hepatic and renal thioredoxin-interacting protein (TXNIP), which is a member of thioredoxin system, were affected in the case of iron overload in the mouse at the protein and gene levels.[5]The increased TXNIP protein expression is a marker for the activated apoptosis pathway.[6] While hepatic TXNIP expression increased with iron overload, its expression decreased in the kidney. According to the results, it might be said that the apoptosis pathway in the liver is triggered by increased expression of TXNIP in the event of iron overload. The question now is how the hepatic and renal glutathione system will act at the protein level during iron overload. To demonstrate the formation of oxidative stress in liver and kidney tissues of mice intraperitoneally injected 5 mg iron dextran solution twice weekly for three weeks, the reduced glutathione (GSH) level, which are the markers of oxidative stress, was spectroscopically analyzed in both tissues. While the GSH level decreased in the liver, its level increased in the kidney after the iron overload. Protein e xpression of TXNIP in iron overload model was examined in both tissues using western blotting. While TXNIP protein expression increased in the liver, its expression decreased in the kidney after the iron overload. Glutathione peroxidase (GPx) and glutathione reductase (GR) enzyme activities were spectroscopically investigated in both tissues. While the enzyme activity of GPx and GR significantly increased in the liver, excess iron didn't cause any change in both enzyme activities in the kidney. In conclusion, iron overload exhibited much stronger adverse effect on the hepatic glutathione system than the renal glutathione system. Because, In the case of iron overload, liver tissue begins to tend to apoptosis via TXNIP.

References:

1) Wang, J.; Pantopoulos, K. *Biochemical Journal*. **2011**, 434 (3), 365-381.
2) Budak, H.; Gönül, N.; Ceylan, H.; Kocpınar, E.F. *Comparative Biochemistry and Physiology.* **2014**, 365–370.
3) Mehta, S. K.; Gowder, S. J. T. *Basic Principles and Clinical Significance of Oxidative Stress*. **2015**, 59-65.
4) Metere, A.; Frezotti, F.; Graves, C. E.; Vergine, M.; De Luca, A.; Pietraforte, D.; Giacomelli, L. *Cancer Cell International*. **2018**, 18(1): 7.
5) Sönmez, F.; Budak, H. *1st Eurasia Biochemical Approaches Techonologies (EBAT)*. **2018,** S-050, page 51.
6) Huang, C.; Zhang, Y.; Kelly, D. J.; Tan, C. Y.; Gill, A.; Cheng, D.; Braet, F.; Park, J. S.; Sue, C. M.; Pollock, C. A. *Scientific Reports*. **2016**, 291-96.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Purification of Carbonic Anhydrase From Alectoris chukar Liver Tıssue And The Determination of Some Biochemical Parameters of Enzyme

Hasan Karagecili[a], Ramazan Demirdag[b], Emrah Yerlikaya[a]

[a]*Siirt University, School of Health, 56100, Siirt, Turkey*
[b]*Agrı Ibrahim Cecen University, School of Health, 04100, Agrı, Turkey*
emrahyerlikaya@siirt.edu.tr

Carbonic anhydrase (CA, E.C. 4.2.1.1) is found nearly in all tissues. The zinc contain enzyme catalyzes the reversible reactions of bicarbonate dehydration and carbon dioxide hydration physiologically[1]. In present study, the carbonic anhydrase enzyme was purified 460.7-folds with 55.5% yield from Alectoris chukar liver tissue by Sepharose-4B-L-tyrosine-sulfanilamide affinity column for the first time. The specific activity was determined as 1.23 EU/mg protein. Alectoris chuckar is an ecologically important species like any living thing. It is one of the main enemies of insects such as Aelia rostrata and Eurygaster integriceps which are harmful for agriculture. In addition, Alectoris chuckar is a species that is highly preferred by hunters. The rhythm of sound is liked by most people. CA enzyme has so far been purified from many species. However, no studies on Alectoris chuckar's have been found in the literature. Some biochemical parameters of enzyme were investigated such as molecular weight, optimum pH, optimum temperature and optimum ionic strength. The molecular weight determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis was found to be about 28.4 kDa. The optimum pH, optimum temperature and optimum ionic strength were determined to be 8.0 Tris–SO_4 buffer, 35°C and 50 mM Tris–SO_4 buffer for the CA enzyme, respectively. K_M and V_{max} values were calculated for NPA by Lineweaver–Burk graph. K_M constants were calculated as 1.035 mM, V_{max} values as 0.02 mol×min^{-1} for NPA.

References :

Caglayan, C.; Taslimi, P.; Turk, C.; Kandemir, F.M.; Demir, Y.; Gulcin, I. Purification and characterization of the carbonic anhydrase enzyme from horse mackerel (Trachurus trachurus) muscle and the impact of some metal ions and pesticides on enzyme activity. Comparative Biochemistry and Physiology, Part C 226. (2019). 108605.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Enzyme Inhibition Activities of the Various Extracts of the Root and Aerial Parts of *Chaerophyllum bulbosum*

Gülsen Tel-Çayan[a], Ebru Deveci[b], Zeynep Molo[b], Mehmet Emin Duru[b], Mehmet Öztürk[b]

[a]*Muğla Sıtkı Kocman University, Muğla Vocational School, Department of Chemistry and Chemical Processing Technologies, Muğla, TURKEY*

[b]*Muğla Sıtkı Kocman University, Faculty of Science, Department of Chemistry, Muğla, TURKEY*

**Corresponding Author: gulsentel@mu.edu.tr*

Enzyme inhibition is an important area of pharmaceutical research because it allows the discovery of a wide variety of drugs useful in many chronic diseases.[1] Inhibition of α-amylase and α-glucosidase are used for the treatment of hyperglycemia (the hallmark of type 2 diabetes mellitus); inhibition of tyrosinase for hyperpigmentation; inhibition of urease for ulcer.[2]

In this study, α-amylase, α-glucosidase, urease and tyrosinase inhibitory activities of the various extracts (hexane, chloroform, acetone, methanol and water) of the root and aerial parts of *Chaerophyllum bulbosum* were spectrophotometrically screened. The highest α-amylase and tyrosinase inhibitory activities were found in the hexane extracts of the root and aerial parts of *C. bulbosum.* Against α-glucosidase, chloroform extracts of *C. bulbosum* root (55.23±0.81 %) and aerial parts (56.94±0.69 %) indicated the best inhibitory activity. The water extracts of the root and aerial parts of *C. bulbosum* showed the highest urease inhibitory activity among the other extracts. Also, the water extract of *C. bulbosum* aerial part exhibited higher urease inhibitory activity than thiourea used as standard with inhibition values of 68.69±0.54 % (at 25 µg/mL concentration) and 89.66±0.95 % (at 100 µg/mL concentration).

Acknowledgment: Authors would like to thank The Scientific and Technological Research Council of Turkey (TUBITAK-118Z610) for financial support.

References:

Amtul, Z.; Atta-ur-Rahman; Siddiqui, R. A.; Choudhary, M. I. *Curr. Med. Chem.* **2002**, *9,* 1323-1348.
Zolghadri, S.; Bahrami, A.; Khan, M. T. H.; Munoz-Munoz, J.; Garcia-Molina, F.; Garcia-Canova, F.; Saboury, A. A. *J. Enzyme Inhib. Med. Chem.* **2019,** *34,* 279-309.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Inhibitory Effects of Some Pesticides And Metals on Carbonic Anhydrase Purified From Alectoris chukar Liver Tıssue

Hasan Karagecili[a], Ramazan Demirdag[b], Emrah Yerlikaya[a]

[a]*Siirt University, School of Health, 56100, Siirt, Turkey*

[b]*Agrı Ibrahim Cecen University, School of Health, 04100, Agrı, Turkey*

hasankaragecili@siirt.edu.tr

Pesticides are commonly known as medium which are used in order to prevent and control of hazardous organisms. They are immensely used in pest control [1]. On the other hand, heavy metals are contaminants for environment and can be described in living systems and ecological. Humans are exposed to them via polluted food, water and air [2]. In this study, *in vitro* inhibitory effects of some pesticides (Spinosad and Dimethoate) and metal ions (Al^{3+}, Cu^{2+}, Ba^{2+}, Fe^{2+}, Mn^{2+}, Se^{2+}) were examined on carbonic anhydrase purified from Alectoris chukar liver tissue. For the compounds showing inhibitory effects, the IC_{50} value was determined by activity% vs inhibitor concentration graphs for each compound. The kinetic parameters of this enzyme were determined for its esterase activity, with 4-nitrophenyl acetate as substrate. IC_{50} values of Al^{3+}, Cu^{2+}, Ba^{2+}, Fe^{2+}, Mn^{2+} and Se^{2+} ions were determined to be 0.29, 2.07, 1.20, 0.34 and 2.69 mM, respectively. It was observed that Spinosad and Dimethoate pesticides inhibited the enzyme at very small concentrations such as 0.37 and 0.58 mM.

References :

Guller, P.; Akkemik, E.; Sevil Kör, S.; Ciftci, M. Investigation of Some Pesticides' Effects on Activities of Glutathione Reductase and Glutathione S-Transferase Purified from Turkey Liver under in Vitro Conditions. Igdır University Journal of the Institute of Science and Technology. 2018.8(3): 211-217.

Caglayan, C.; Taslimi, P.; Turk, C.; Kandemir, F.M.; Demir, Y.; Gulcin, I. Purification and characterization of the carbonic anhydrase enzyme from horse mackerel (Trachurus trachurus) muscle and the impact of some metal ions and pesticides on enzyme activity. Comparative Biochemistry and Physiology, Part C 226. (2019). 108605.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effects of Nitrogen Rich Energetic Compounds on Microbial Growth

S.Betül Sopacı

Ahi Evran Üniversitesi Fen Edebiyat Fakültes, Kimya Bölümü, Biyokimya Anabilimdalı Bağbaşı Kampüsü 40080 Kırşehir
bsopaci@ahievran.edu.tr

Aromatic nitro compounds are well recognized as important energetic materials which are used in industry and military purposes. Synthesis and analysis of these materials are gaining attention because of their utility in various applications as explosives , pharmaceuticals and pyrotechniques. There are some known drugs and drug candidates including antimicrobial agends, containing aromatic or heterocyclic derivatives of energetic nitro compounds. In addition to this, their biological activities are of interest for their biotransformation to less toxic compounds. Manufacture of nitro-substituted explosives, testing and firing ranges, and destruction of ammunition stocks have generated toxic wastes, leading to large-scale contamination of soils and groundwater.[1] In this context we are presenting a report about effects on microbial growth of wide range of newly synthesized nitrogen rich energetic compounds. *Echerichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Entherococcus faecalis, Staphylococcus enteritidis, Bacillius licheniformis and Bacillus subtilis* were the bacterial species that have been tested against our energetic compounds and we obtained significant results on bacterial growth with respect to toxic (bactericidal and bacteriostatic) and stimulant effects. According to the results some explosives were shown toxicity at 4 ppm, and some stimulate the growth around 100 ppm concentrations. Results were also evaluated as the chemical structure and concentration (MIC) of the compounds and Gram state of the microorganisms.

References

Spain, J. C. Biodegradation of nitroaromatic compounds and explosives: introduction, p. 1–6. In J. C. Spain, J. B. Hughes, and H. J. Knackmuss (ed.), Biodegradation of nitroaromatic compounds and explosives. Lewis Publishers, New York, N.Y., **2000**.

References (optional):

Kalin, J. H.; Bergman, J. A. *J. Med. Chem.* **2013**, *56*, 6297.
Molyneux P., Water-soluble Synthetic Polymers: Properties and Behavior, 2nd Ed., CRC Press, Boca Raton, Florida, 1984.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Determination of Antioxidant Properties of Lacmoid

Fevzi Topal[a], Meryem Topal[b] İlhami Gülçin[c]

[a]*Gumushane Vocational School, Department of Chemical and Chemical Processing Technologies, Laboratory Technology Program, Gumushane University, Gumushane, Turkey*
[b]*Gumushane University, Vocational School of Health Services, Gumushane, Turkey*
[c]*Department of Chemistry, Faculty of Science, Atatürk University, Erzurum, Turkey*
topalfevzi@hotmail.com

Lacmoid is a small organic molecule derived from resorcin (1,3-dihydroxybenzene) by heating sodium nitrite at 120°C.[1] It interfere with the oligomerisation or fibrillation processes of proteins related to neurodegenerative diseases. To determine its radical scavenging and antioxidant activities, diverse *in vitro* methods such as $ABTS^{\bullet+}$ scavenging, $DPPH^{\cdot}$ scavenging, $DMPD^{\bullet+}$ scavenging, Cu^{2+} reduction (CUPRAC), Fe^{3+}-TPTZ reduction (FRAP), Fe^{3+} reduction and Fe^{2+} chelating activities were used and compared to the standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol and Trolox.[2,3] BHA, BHT, α-tocopherol, Trolox and Lacmoid displayed 3.16, 3.18, 3.65, 38.5 and 5.82 µg/mL $ABTS^{\bullet+}$ scavenging activity, respectively. The results of this study clearly showed that lacmoid demonstrated the most powerful antioxidant activity and radical-scavenging activity. Also, these results will guide future studies for lacmoid.

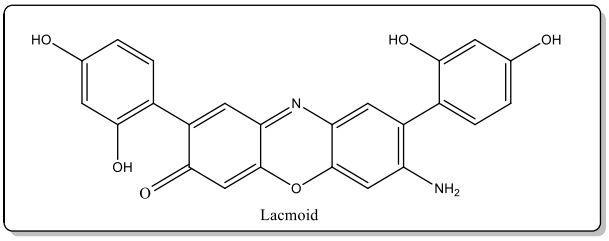


Figure 1. The structure of lacmoid

References:

Ellms, J. W. *J. Am. Chem. Soc.***1899,** 21, 359-369.
Abelein, A., Lang, L., Lendel, C., Graslund, A., Danielsson, J., *FEBS Lett.* **2012**, 586, 3991-3995.
Gulcin, I. *Arch.Toxicol*. **2012**, 86, 345-391.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Immobilization Of Arginase On Cellulose Acetate (CA)/Polyvinylpyrrolidone (PVP)/Mn^{+2} Electrospun Nanofibers With Efficient And Effective Stability Properties

Ceyhun Işık[a], Mustafa Teke[a]
[a]*Department of Chemistry, Muğla Sıtkı Koçman University, Muğla, Turkey.*
cyhn87@yahoo.com

Arginase (EC 3.5.3.1) is a manganese-metalloenzyme which irreversibly catalyzes the hydrolysis of L-arginine into L-ornithine and urea. It has recently been considered not only as an analytical tool for L-arginine assays but also as a prospective pharmaceutical in enzyme therapy of cancers auxotrophic for arginine[1].

Enzyme immobilization has a wide working area in clinical and industrial aspects. Immobilized enzymes have most advantages such as repeated use of the enzyme, inhibition of the enzyme by matrix, inhibition of contaminating with the product, resistance to effects such as pH and temperature, and the possibility of achieving more than one reaction at the same time[2].

In this submitted work, arginase was immobilized onto CA/PVP/Mn^{+2} nanofibers which were synthesized by electrospinning technique. The most appropriate operational parameters as electric voltage, distance between tip and collector, concentration of CA, concentration of PVP, concentration of Mn^{+2}, injection speed were found as 17 kV, 15 cm, 15% CA, 5% PVP, 0.1% Mn^{+2} and 0.6 ml/h respectively. The morphology and structure of the nanofibers was characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR) and thermal gravimetric analysis (TGA). According to our results, CA/PVP nanofibers were successfully synthesized by electrospinning technique. And then arginase was immobilized on to the nanofibers by adsorption and crosslinking methods. For the optimization of arginase immobilization, the amount of nanofiber (12.5 mg), the adsorption time (15 min), the unit of arginase (0.5 U/ml) and the amount of glutaraldehyde (5%) were determined as basic parameters. The optimum temperature, optimum pH, thermal stability, pH stability, kinetic parameters and reusability parameters were investigated in the characterization of arginase immobilized nanofibers prepared under optimum conditions.

The significance of this study is improving of stability properties of arginase for various biotechnological applications such as the production of L-ornithine and promising approach to control cancer.

References:

Johnny, M.; Corine, G.; Celine, D. *Experimental Gerontology*, 2019, 116, 54-73
Zhen, G. W.; Ling, S. W.; Zhen, M. L.; Xiao, J. H.; Zhi, K. X. *J. Mol. Catal. B Enzym.* 2009, 56 (4), 189-195

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Anticholinesterase and Anti-tyrosinase Activities of Isolated Compounds from *Fuscoporia torulosa*

Ebru Deveci[a,b*], Gülsen Tel-Çayan[c], Mehmet Emin Duru[a]

[a]*Muğla Sıtkı Kocman University, Faculty of Science, Department of Chemistry, Muğla, TURKEY*

[b]*Konya Technical University, Technical Sciences Vocational School, Chemistry and Chemical Processing Technology Department, Konya, TURKEY*

[b]*Muğla Sıtkı Kocman University, Muğla Vocational School, Department of Chemistry and Chemical Processing Technologies, Muğla, TURKEY*

**Corresponding Author: ezengin@mu.edu.tr*

Mushrooms are important sources of natural bioactive compounds. Until now, primary and secondary bioactive compounds with immunomodulator, anti-inflammatory, analgesic, chemopreventive, anti-tumor, chemo and radio protective, antibacterial, anticholinesterase, antiviral, anti-diabetic, anti-angiogenic, antioxidant and anti-ulcer properties have been obtained from mushrooms.[1]

In this study, a new steroid (**1**) and ten known (**2-11**) compounds were isolated from *Fuscoporia torulosa* mushroom. Their structures were elucidated as 5*α*,8*α*-epidioxyergosta-6,22-dien-3*β*-il-palmitate (**1**), ergosta-4,6,8(14),22-tetraen-3-one (**2**), ergosterol peroxide (**3**), oleanolic acid (**4**) 28-norolean-12-en-3*β*-ol (**5**), javeroic acid (**6**), β-sitosterol (**7**), oleanonic acid (**8**), 2,3-dihydroxy cinnamic acid (**9**), 3,4-dihydroxy benzaldehyde (**10**), 4-(3,4-dihydroxyphenyl)but-3-en-2-one (**11**) on the basis of spectroscopic analyses (IR, NMR and MS) and comparison with literature data. Anticholinesterase and anti-tyrosinase activities of all isolated compounds were determined. When compound **4** (29.19±0.17 %) showed the highest anti-AChE activity, compound **8** (67.40±0.33 %) exhibited the highest anti-BChE activity. Among all isolated compounds, compound **11** (21.09±0.23 %) was found to be the most active against tyrosinase.

Acknowledgment: Authors would like to thank The Mugla Sitki Kocman University Research Fund (MUBAP 15/238) for financial support.

References:

Wasser, S. P. *Int. J. Med. Mushrooms*. **2010,** *12(1),* 1-16.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation Effects of Deinoxanthin on Gene Expression and Activity of Separase in Hepatocellular Carcinoma HepG2 Cells

Mehmet KUZUCU

Erzincan Binali Yıldırım University, Faculty of Arts and Sciences, Department of Biology, Erzincan, Turkey

mkuzucu@erzincan.edu.tr

Separase is a cysteine protease which hydrolyzing cohesin ring complex releasing the tension of the spindle and allowing segregation of sister chromatids to opposite centrosomal poles. Upregulation of Separase has been is defined as lagged chromosomes, premature separation of chromatids and aneuploidy. Disrupt mitosis and cell cycle regulation can result in cancer formation[1,2]. Separase associated with the development and progression of human hepatocellular carcinoma[2].

Deinoxanthin is a highly pro-apoptotic carotenoid derivative found in the cell wall of *Deinococcus radiodurans*, the most radiation-resistant organism in the world. Cell culture studies have shown that deinoxanthin induces apoptosis on hepatocellular, colon and prostate cancers. Deinoxanthin has a higher antioxidant effect than other carotenoids (lycopene, beta-carotene, zeaxanthin) and therefore it has a higher ROS clearance ability[3].

In the present research was to examine effects of the Deinoxanthin on gene expression and activity of Separase in hepatocellular carcinoma, HepG2 cells. It has been studied that the gene expression level of ESPL1 with the human β-actin used as a housekeeping-gene to normalize data. Furthermore, the activity of Separase in the deinoxanthin-applied cells by fluorogenic assay method using Rad21-MCA peptide (Ac-Asp-Arg-Glu-Ile-Nle-Arg-MCA) as substrate.

References:

Gurvits, N., Löyttyniemi, E., Nykänen, M., Kuopio, T., Kronqvist, P., & Talvinen, K. (2017). Separase is a marker for prognosis and mitotic activity in breast cancer. British journal of cancer, 117(9), 1383.

Ricke, R. M., van Ree, J. H., & van Deursen, J. M. (2008). Whole chromosome instability and cancer: a complex relationship. Trends in genetics, 24(9), 457-466.

Choi, Y. J., Hur, J. M., Lim, S., Jo, M., Kim, D. H., & Choi, J. I. (2014). Induction of apoptosis by deinoxanthin in human cancer cells. Anticancer research, 34(4), 1829-1835.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

IN VITRO/IN VIVO/EX VIVO EVALUATION ANTI-CANCER POTENTIAL OF A NOVEL SECOND GENERATION PLATINUM DERIVATIVE

Habibe Yılmaz[1,2], Şenay Hamarat Şanlıer[1,2]

[1] *Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*
[2] *Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*

yilmaz.habibe@hotmail.com

Abstract

Platinum (Pt) -based anticancer agents are the mainstay of clinical drugs used to treat various solid tumors such as genitourinary, colorectal and non-small cell lung cancer. The leading anticancer drug, cisplatin, has been used in standard chemotherapy regimens as a single treatment for more than thirty years, or in combination with other cytotoxic agents or radiotherapy.

The aim of the study is the evaluation of solid cancer treatment potenial of a novel second generation active platinum derivate. Cytotoxicity assay performed on SKOV3-Luc and A549-Luc cell lines. Treatment potential was evaluated in xenograft tumor models. Biodistribution was tested on tumor-bearing mice via Pt analysis of organs with ICP-MS. Cis-4-hydroxyphenylplatinum(II)diamine compound was synthesized with a yield of 62%. The MTT assay on the A549-Luc and SKOV3-Luc cell lines resulted in IC50 values of 13.44 and 7.91 µM at 72h, respectively. Tumor growth continued in the control group when healing was observed in the treatment group. As a conclusion, the compound has a treatment potential.

Keywords: Platinum-derivative, anti-cancer agent, xenograft model, biodistribution, lung cancer, in vitro assay.

Small and Large Scale Solid Phase Peptide Synthesis (SPPS) at Elevated Temperatures: Advances, Process Development, and Considerations

Giorgio Marini

Method development for further advancing the efficiency of SPPS is of the utmost importance. Microwave irradiation provides simplified optimization, higher peptide purity, and an overall "greener" process. Compared to conventional heating methods, microwave irradiation provides rapid and direct energy exchange with the reagents.

Our previous research improved coupling efficiency and speed. The result, difficult and long sequences are effectively synthesized in a fraction of the time using much less solvent. Advances have been made which further reduce the cycle time to under 4 min, offer an overall solvent reduction greater than 90 % compared to other SPPS processes, and are readily scalable to generate up to 200 mg purified peptide.

Rapid scale-up for clinical trials and peptide production has been accomplished using similar technology. Crude purity from R&D to production scale is preserved if not improved and unwanted side reactions such as epimerization and aspartimide formation are easily controlled. The result, easier purification and reduced labor cost.

More recently, the usage of a large microwave cavity (up to fifty liter capability) allows us the possibility to scale up laboratory conditions in order to make bigger amount of peptides still taking advantages of the benefits of microwave technology. Cycle times at the production scale range from 10 – 60 min with the capability to produce up to 1 KG crude peptide in a single batch with considerably shorter synthesis time compared to the conventional methods at room temperature.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Interaction of L-T3 with Zwitterionic Dipalmitoyl Phosphatidylcholine Model Membranes

Abeer Alesknd rany, Ipek Sahin

Department of Physics, Institute of Science, Ege University, 35100 Bornova-Izmir, Turkey

aalesknd rany@gmail.com

T3 (triiodothyronine), an active form of thyroid hormone (TH), plays a crucial role in body control, including growth and development, metabolism, body temperature, and heart rate. It differs from T4 in terms of three iodine atoms instead of four and is derived primarily from T4 through deiodination outside the thyroid gland. L-T3 (levotriiodothyronine) is a synthetically produced form of T3. It used to treat hypothyroidism (low thyroid hormone) and goiter (enlarged thyroid gland). It is given as part of medical tests for thyroid disorders.

This study aimed to evaluate the temperature and concentration-induced effects of L-T3 on zwitterionic dipalmitoyl phosphatidylcholine (DPPC) model membranes using two noninvasive techniques, namely Fourier Transform Infrared (FTIR) Spectroscopy and Differential Scanning Calorimetry (DSC). FTIR spectroscopy and DSC were applied to investigate the interactions of L-T3 with DPPC multilamellar liposomes (MLVs) as a function of temperature and low (3 mol %) and high (15 mol %) concentrations of L-T3. The investigation of the C-H, C=O, and PO_2^- antisymmetric double stretching modes in FTIR spectra and DSC studies reveal that the inclusion of L-T3 changes the physical properties of the DPPC MLVs.

The DSC peaks broaden and shift to lower temperature degrees with increasing concentrations of L-T3. The pretransition of liposomes is eliminated for all samples containing L-T3. At high concentration of L-T3, the curve contains more than one peak indicating the existence of phase separation. FTIR results show that the main transition temperature of DPPC decreases and the phase transition curve broadens with the addition of L-T3. While both concentrations of L-T3 order the system in the gel phase, low concentration disorders it in the liquid crystalline phase. Furthermore, L-T3 increases the dynamics of the acyl chains of DPPC both in the gel and liquid crystalline phases. L-T3 also induces a decrease in the wavenumber values of the C=O stretching and PO_2^- antisymmetric double stretching bands of DPPC both in the gel and liquid crystalline phases, pointing out the hydrogen bonding in between the hydroxyl group of L-T3 and the carbonyl and phosphate groups of DPPC membranes.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Fe_3O_4 Nanoparticle Supported Poly(Methylene Blue) Modified Gold Electrode as H_2O_2 Sensor

Ahmet KARAÇELİK[a], Okan UÇARLI[b], Saim TOPÇU[a]

[a]*Giresun University, Science and Arts Faculty, Department of Chemistry,Giresun,Turkey*
[b]*Giresun University, Espiye Vocational School, Medical Services and Techniques Department, Giresun,Turkey*
e-mail:karacelik_ahmet@hotmail.com

A strong oxidant agent hydrogen peroxide formed in biochemical processes is a vital analyte in terms of health. When a Poly(Methylen Blue), a conductive polymer, coated Gold Electrode decorated with heterogeneous catalyst Fe_3O_4 nanoparticles, electrode shows an electrochemical signal at about -850 mV for low analyte concentrations.[1,2] The polymer synthesized with 20 cycles in the range of -600 - 1150 mV was determined to be the most suitable modification thickness. Furthermore, the electrochemical activation of the gold electrode surface before processing improved the performance of the electrode. As a result of each procedure to monitor the modification stages of the electrode, Electrochemical Impedance Spectrums were taken and analyzed. The resulting spectra were fitted to Randles equivalent circuits, and the changes were quantitatively determined.

Optimal conditions for pH value where the conductive polymer is synthesized to be 6.5 and phosphate buffer pH 7.0 for peroxide analysis have been determined. It was determined by linear regression of the resulting signals that the optimum concentration range was 2 - 20 mM in these conditions.

References:

Silber, A.; Hampp, N.; Schuhmann, W. Biosensors and Bioelectronics. **1996,** 215-223.
Zhao, G.; Feng J.J.; Zhang, Q.L.; Li, S.P.; Chen, H.Y. Chem. Mater. **2005,** 3154-3159.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Synthesis of Calix[4]arene Derivatives Carrying L-Proline Groups and Their Cytotoxic Properties and Effects on DNA Structure with Various Human Cancer Cell Lines

Alev GÜL[a], Mehmet OĞUZ[a,b], Serdar KARAKURT[c], Mustafa YILMAZ[a]

[a]Selcuk University, Department of Chemistry, 42075 Konya, Turkey

[b]Department of Advanced Material and Nanotechnology, Selcuk University, 42075 Konya, Turkey

[c]Selcuk University, Department of Biochemistry, Konya 42075, Turkey

alevgul52@gmail.com

Calix[n]arenes synthesized by activation of phenol formaldehyde under appropriate conditions have been subject to many researches because of having ring structure which easily and limitless functioned from either phenolic-O-position or p-positions. Since calix[n]arenes have basket structure, they have been used to carry many ions and molecules[1]. Recently, some calix[n]arene derivatives have been studied for their capacity on drug solubility and biochemical studies. Beside this, in the last decades, anti-cancer properties proved some calix[n]arene derivatives were used for phase studies. Cancer is the uncontrolled and abnormal growth of the mechanisms that regulate the normal behavior of cells. Now of the important health problems resulted with that is cancer especially in determined cell. The aim of the present study is the synthesis of the upper rim and lower rim-functionalized L-proline-based calix[4]arene derivatives and evaluation of their cytotoxic potential for human cancerous cells as well as to determine the death mechanism[2]. Synthesized calix[4]arene (3 and 8) derivatives were characterized by different spectroscopic techniques such as ^{1}HNMR, ^{13}CNMR and FTIR. For that purpose proline containing calix[4]arene was synthesized and its effect on human prostate, colon, lung and liver cancer was investigated according to Alamar Blue test. Besides this, DNA damage and apoptosis were investigated by using follow cytometer. *In vitro* effects of compounds 3 and 8 were tested on human cancerous cells (HEPG2, PC-3, A-549, and DLD-1) as well as human healthy epithelium cell (PNT1A).

Acknowledgments: The authors are indebted to the Scientific and Technological Research Council of Turkey (TUBİTAK, Grant No:116Z173) for their financial support of this work.

References (optional):

Yilmaz M., Sayin S. Calixarenes in Organo and Biomimetic Catalysis. In: Neri P., Sessler J., Wang MX. (eds) Calixarenes and Beyond. Springer, Cham, 2016 pp 719-742.

Oguz M., Gul A., Karakurt S., Yilmaz M., Synthesis and Evaluation of the Antitumor Activity of Calix[4] arene L-Proline Derivatives, Bioorganic Chemistry, 2019, 103207.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Determination of Antioxidant and Antimicrobial Activities of Naturally Growing Salmanca Plant

Ayça Aktaş Karaçelik [a], Hüseyin Şahin[a], Ömer Ertürk[b]

[a] *Giresun University, Espiye Vocational School, Giresun, Turkey*

[b]*Ordu University, Faculty of Arts and Sciences, Department of Biology, Ordu,Turkey*

e-mail: aycaaktas84@hotmail.com

In recent years, herbal products have been gaining interest worldwide, especially in various diseases, and in order to provide better living conditions, scientific research on the subject has accelerated with developing technology. Especially green leafy vegetables are seeing widespread interest as they offer many benefits for human health.[1] Salmanca plant is located under the genus Chenopodium (*Chenopodiaceae*), which is distributed worldwide and contains about 250 species. This vegetable has traditionally been used as a blood purifier, diuretic, sedative drug, liver protector, laxative and effective against roundworms and hairworms.[2] In order to shed more light to bioactivity of salamanca, antioxidant and antimicrobial activities of the plant's extracts by using different solvents (methanol, acetonitrile and water) were determined.

DPPH and ABTS radical scavenging activities, ferric reducing antioxidant power (FRAP) and total phenolic contents determination were used to evaluate the antioxidant activity of the extracts. The results were compared with standard antioxidants BHT, Trolox, gallic acid and quercetin. Antimicrobial activity was determined by diffusion test and minimum inhibition concentration (MIC) test. The antimicrobial activities of the extracts were tested against 10 bacteria (*E. coli*, *C. freundii*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *B. Subtilis, Y. enterocolitica, B. ceraus, C. albicans, E. fecalis*).

There was a positive correlation between antioxidant activity results. Accordingly, the highest antioxidant activity was detected in methanol extract, and the lowest activity was detected in aqueous extract in all tests. According to antimicrobial results, all extracts were not examined as effective when compared with standard antibiotics (ampicillin, cefazolin, nystatin), but acetonitrile and methanol extracts of salmanca could be considered more preferable than aqueous one. In the light of these studies, we believe that Chenopodium extracts can be used as an easily accessible natural antioxidant source and will play an important role in the production of food supplements and pharmaceutical industry.

*This study was supported by Giresun University Research Fund, Scientific Research Projects, Project FEN-BAP-A-150219-17.

References:

Peterson J., M.S.; Dwyer J., D. S., *Nutrion Research*, **1998**, 18, 1995-2018.

Kokanova-Nedialkova, Z.; Nedialkov, P. T.; Nikolov, S. D., *Phcog Rev*,**2009**, 280-306.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Extracellular-Matrix Like Multicomponent Hydrogels For Complex Bone Tissue Engineering

Burak Derkus[a,b,c], Alvaro Mata[a,b]

[a]*Institute of Bioengineering, Queen Mary University of London, E1 4NS London, UK.*

[b]*School of Engineering and Materials Science, Queen Mary University of London, E1 4NS London, UK.*

[c]*Biomedical Engineering Department, Faculty of Engineering, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey.*

burakderkus@gmail.com

The inherent extracellular matrix (ECM) is a complex gel-like construct with fibrous architecture. The fate and functions of cells, particularly stem cells, strongly hinge on the chemical and physical (stiffness) properties of native ECM. Therefore, designing complex biomaterials that recapitulate the complexity of native ECM is a potential strategy to create synthetic tissue analogues and to mimic ECM. Multicomponent co-assembly is a strategy which proposes simple, scalable, multifunctional, approach to fabricate complex hydrogel-based tissue environments. Therefore, in this study we harnessed the simplicity of multicomponent co-assembly to fabricate complex hydrogels incorporating synthetic bioactive tools (peptide amphiphiles, PAs) that promote cell adhesion, pro-angiogenesis, and osteogenesis in both 2D and 3D. We confirmed cell adhesion, spreading, and differentiation both on and within the hydrogels. Finally, we confirmed bone-vascular hybrid co-culture and bone-like construct formation from hAMSCs and HUVECs in 2D and 3D co-culture systems using immunofluorescent staining and real-time quantitative polymerase chain reaction (RT-qPCR). The designed ECM-like multicomponent hydrogels hold great potential as functional scaffolds for complex-bone tissue regeneration.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Comparison of the Effect of Morin on Xenobiotic Metabolizing Enzymes in Healthy and Streptozotocin-induced Diabetic Rats Treated with 7,12-Dimethylbenz[a] anthracene and Endosulfan

Canan Sapmaz[a] , Azra Bozcaarmutlu[a], Aysel Kukner[b,c], Tulin Firat[b]

[a] *Bolu Abant Izzet Baysal University, Faculty of Arts and Science, Department of Chemistry, Bolu, Turkey*

[b] *Bolu Abant Izzet Baysal University, Faculty of Medicine, Department of Histology and Embryology , Bolu, Turkey*

[c] *Near East University, Faculty of Medicine, Department of Histology and Embryology, Lefkosa, North Cyprus*

sapmaz_c@ibu.edu.tr

Morin (M) is a dietary flavonoid, abundant in almond, onion, seed weeds, fustic, guava, Osage orange, apple and red wine. In this study, its effect was compared in the presence of toxic chemicals, 7,12-dimethylbenz[a]anthracene (A) and endosulfan (E) on cytochrome P450s associated enzyme activities (7-ethoxyresorufin O-deethylase (EROD), 7-methoxyresorufin O-demethylase (MROD), 7-pentoxyresorufin O-dependentylase (PROD), aniline 4-hydroxylase (A4H), erythromycin N-demethylase (ERND)), total glutathione S-transferase (GST) and glutathione reductase (GR) enzyme activities in healthy and diabetic (D) rats. For this purpose, the rats were randomly selected and separated into sixteen groups (C (control), E, M,E+M, A, A+M, A+E, A+E+M, D, D+E, D+M, D+E+M, D+A, D+A+M, D+A+E, D+A+E+M). Morin (25 mg/kg body weight (bw) in water, three times per week), endosulfan (5 mg/kg bw endosulfan in corn oil, three times per week), and DMBA (30 mg/kg bw in corn oil, once per week for three week) were administered either individually or in combinations to rats. In diabetic groups, diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg bw). The rats were killed by cervical dislocation on day 54 (healthy groups) and on day 43 (diabetic groups). Administration of E+M, A+M and A+E+M significantly increased EROD activity in healthy rats compared to C. MROD and GST activities of healthy rats treated with A+M and A+E+M were significantly higher than rats in C group. In diabetic rats, co-administration of A and M significantly decreased the elevated EROD activities of diabetic rats treated with A. Similar to EROD activity, A+M treatment decreased MROD activities of diabetic rats compared to A group, however this decrease was not significant. M and A+M administration significantly decreased GST activity compared to D group in diabetic rats. Co-administration of A and M did not alter the PROD activities of healthy rats compared to group A, however this activity decreased in diabetic rats. Significant increases were observed in ERND and A4H activities in healthy rats with the co-administration of A+E+M. In diabetic rats, co-administration of A and M significantly decreased A4H activities compared to D group. Although A+M administration significantly increased GR activities in healthy rats, it did not alter GR activity in diabetic rats. The results of this study indicate that diabetic individuals give different responses to morin in the presence of toxic chemicals in xenobiotic metabolizing enzyme activities compared to healthy individuals.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Biocompatibility of Castor Oil and Bacterial Cellulose Based Thermoresponsive Hydrogels

Elif Isikci Koca[a], Gulnihal Bozdag [b], Ozlem Yalcin Capan[c], Gokhan Cayli[d], Dilek Kazan[b], Pinar Cakir Hatir[a]

[a]Biomedical Engineering Department, Istanbul Arel University, Istanbul, Turkey

[b]Bioengineering Department, Marmara University, Istanbul, Turkey

[c]Department of Molecular Biology and Genetics, Istanbul Arel University, Istanbul, Turkey

[d]Department of Engineering Sciences, Istanbul University-Cerrahpasa University, Istanbul, Turkey

elifisikci@arel.edu.tr

Hydrogels are three-dimensional hydrophilic polymeric networks which swell in aqueous environments. Thermoresponsive hydrogels are one of the important biomaterials employed in many biomedical applications especially in controlled drug release systems as they have abilities to respond to external temperature changes. [1] This study focuses on the investigation of biocompatibility of castor oil (CO) and bacterial cellulose (BC) based thermoresponsive hydrogels. CO, one of the vegetable oils, is obtained directly from the plant source. [2] BC is also one of the renewable materials that have flexible, elastic and biodegradable structures. [3] High water retention and chemical modification capacity make BC an ideal material for biomedical engineering applications.

In the present study, novel thermoresponsive hydrogels were synthesized from renewable materials. Three different hydrogels were obtained by two-step polymerization. The first step of the polymerization involves the reaction between BC, CO and 4,4'-diphenylmethane diisocyanate (MDI), whereas in the second step N-isopropylacrylamide (NIPAM) was introduced to the polymers. After having the hydrogels, biocompatibility behaviors were investigated by MTT assays. Cytotoxicity tests were performed with monomer concentrations from 1 mg/mL to 10 mg/mL and from 1 mg/mL to 20 mg/mL of the hydrogel concentrations. Cytotoxicity assays showed at least 50% of cell viability in the presence of 1 mg/mL of each monomer solution, except MBAM, whereas the cell viabilities were above 65% for 1 mg/mL concentrations of the hydrogels.

The authors are grateful to the Marmara University for financial support under Bapko Project, No. FENC-DRP-170118-0011. The authors are grateful to Mehmet Kıranşal and Beyza Kaba for their help with performing MTT assays.

References:

Klouda, L. Eur. J. Pharm. Biopharm. 2015, 97, 338.

Das, S.; Pandey, P.; Mohanty, S. and Nayak, S.K. Polymer Plast Tech Eng. 2017, 56(14), 1556.

Akoğlu, A., Karahan, A.G.M., Çakmakçı, L. and Çakır, İ. Gıda. 2010, 35(2), 127

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Heterologous Expression of a New Exo-Polygalacturonase from *Sporothrix schenckii* 1099-18 in *Pichia pastoris*.

Ersin KARATAŞ[ac*], Ahmet TÜLEK[ac], Barış BİNAY[bc**]

[a] *Gebze Technical University Department of Molecular Biology and Genetics Gebze Kocaeli TURKEY*
[b] *Gebze Technical University Department of Bioengineering Gebze Kocaeli TURKEY*
[c] *Enzim Test ve Danışmanlık Merkezi Gebze Technical University Gebze Kocaeli TURKEY*
**Presenting Author a.tulek@gtu.edu.tr*
*** Corresponding Author binay@gtu.edu.tr*

Pectinolytic enzymes, widespread in plants, fungi and microorganism, gain commercial importance for improving the yield and nutraceutical properties of juice processing industry, degumming of fibre plants and maximum oil recovery. These enzymes break down complex polysaccharide polymers of plant tissues into simpler monomers D-galacturonic acids. It is essentially a hydrolase and based on the mode of action it is further classified as Endo-PG (Polygalacturonase) and Exo-PG. Endo-PG hydrolyses PGA (Polygalacturonic acid) in a random fashion and liberates saturated oligogalacturonides and galacturonic acid. Endo-PGs are produced by numerous fungi and yeast, higher plants and some phytoparasitic nematodes. Exo- PGs catalyze the hydrolytic release of one saturated galacturonic acid residue from nonreducing end of homogalacturonan and are produced by bacteria and fungi.

Since microbial Exo-PGs have immense application in industries, need to identify new sources of this enzyme with properties suitable for different applications and to develop simple procedures for the purification of these enzymes. Hence, in this study we heterologously-expressed a new exo-polygalacturonase from *Sporothrix schenckii* in *Pichia pastoris*.

For this purpose, we isolated Exo-PGs gene from *Sporothrix schenckii* 1099-18 and transformed it into *Escherichia coli* TOP10 cells via pPIC9K vector. After plasmid isolation Exo-PG containing pPIC9K expressed in *Pichia pastoris*. The enzyme has a molecular mass of 52 kDa. Effect of pH on the activity of Exo-PG was studied to determine the optimum pH range. The effect of temperature on the activity was detected to determine the thermostability of the enzyme. Purified enzyme was stored at different temperatures in order to determine the best storage condition. Kinetic analysis of Exo-PG activity was done to detect enzyme's affinity. PGase showed a higher affinity towards PGA than citric pectin. Consequently, the enzyme we discovered is an exo-PGase, EC 3.2.1.82.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Electrochemical Performance of Prussian blue-based hybrid flexible thin films for phosphate anion sensing applications

Esin Eren[a,b], Aysegul Uygun Oksuz[b]

[a]*Department of Energy Technologies, Innovative Technologies Application and Research Center, Suleyman Demirel University, 32260, Isparta, Turkey*
[b]*Department of Chemistry, Suleyman Demirel University, Faculty of Arts and Science, 32260 Isparta, TURKEY*

e-mail: esineren@sdu.edu.tr

Prussian blue (PB) is one of the most successful and frequently used compound in various applications such as sensors, biosensors, electrochromic and electrocatalytic electrodes due to having high electrocatalysis and excellent electrochemical behaviors[1,2,3]. Nickel oxide-based nanoparticles can be offered as a perfect platform for sensing applications due to low cost, high chemical durability, biocompatibility[3]. Recently, flexible applications have gained the escalating demands due their lighter weight, improved comport[4].
In this study, prussian blue-based hybrid flexible thin films containing nickel element were prepared using electrochemical deposition method onto the indium tin oxide (ITO) coated PET electrodes. The hybrid films were characterized using scanning electron microscopy (SEM)-energy dispersive X-ray spectroscopy (SEM-EDS). The electrochemical sensing of the hybrid flexible thin films was evaluated in the presence of different concentrations of phosphate buffer using cyclic voltammetry (CV) method. To investigate the mechanical stability of the flexible hybrid film, a bending cycle test was also conducted.

References:

Hostert, L.; Alvarenga, G.d.; Marchesi L.F.; Soraes A. L.; Vidotti, M. *Electrochimica Acta* **2016**, 213, 822.
Qian, J.; Ma, D.; Xu, Z.; Li, D.; Wang J. *Solar Energy Materials and Solar Cells* **2019**, 177, 9.
Roychoudhury A.; Basu Suddhasatwa B.; JHa S.K. *Analytical Science* **2018**, 34, 1163.
Eren, E.; Alver, C.; Yurdabak Karaca,G.; Uygun, E.; Uygun Oksuz, A. *Synthetic Metals* **2018**, 235, 115.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation Of DNA Damage In Patients Diagnosed With Colorectal Cancer And Investigation Of The Repairing Role Of Beta Glucan On DNA Damage

Necla Benlier, Nilay Uçar, Havva Yeşil Çınkır, Mustafa Yıldırım, Pınar Günel Karadeniz, Eyüp İlker Saygılı,

SANKO University
nucar@sanko.edu.tr

Various techniques can be used to accurately measure the DNA damage. One of these methods is Single Cell Gel Electrophoresis (SCGE), also known as " The Comet Assay Method". This technique; is a non-invasive, fast, sensitive and simple fluorescence microscopic method used to detect the DNA damage in many living species. The Comet Assay method is preferred in apoptosis, aging, clinical and genetic toxicology, oxidative stress-antioxidant studies. Beta glucan is a simple polysaccharide that strengthens the immune system, has a stimulating effect and creates an antioxidant effect. In this study, whole blood samples obtained from patients diagnosed with colorectal cancer (CRC) were investigated by using Comet Assay method to determine the DNA damage and the restorative role of beta glucan ex-vivo in repairing the DNA damage. A total of 19 adult female and male patients (40-80 years old) with a diagnosis of stage 3-4 colorectal cancer who had not started treatment and a healthy control group consisting of 20 female and male (40-80 years old) were included in the study. The whole blood samples taken from the patients (before starting treatment) and the control group were determined the DNA damage (percentage of tail DNA, tail moment and tail length parameters) by Comet Assay method and compared. Then, 50 µl / ml beta glucan was applied ex vivo to the blood samples taken from the patients and the repairing effect of the DNA damage was examined. The images were then taken on a fluorescence microscope and photographed and the degree of the DNA damage was determined.

Before the beta glucan administration in the patient group, while the tail moment was 17,20 ± 8,25, the tail DNA % was 39,25 ± 11,73%, tail length was 37,22 ± 12,25; After application, tail moment was 3.55 ± 2.37%, tail DNA% 8.41 ± 0.54, and tail length 19.72 ± 13.15. In our study, it was found that beta glucan significantly decreased the DNA tail length, the DNA tail percentage and tail moment in ex vivo conditions in CRC patients ($p < 0.001$). In addition, in another comparison between patients who administrated with beta glucan and control group, the tail moment was 11.95 ± 5.65 in the control group and the DNA % in the tail was 26.99 ± 9.75 in the control group. It was found that beta glucan decreased the tail moment and % DNA in the tail statistically. There was no difference in tail length in both groups. Tail length was 18.63 ± 8.20 in the control group. ($P = 0.761$). Tail lengths were not expected to be different from the control group and the patient group ($p < 0.001$). We can conclude that beta glucan is a potent antioxidant, significantly reduces the DNA damage. However, in order to support the results of this study, it may be suggested that new studies with different concentrations of beta glucan in different periods can be suggested.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Immobilization of Trypsin on PVA-Coated Magnetic Nanoparticles

Selmihan ŞAHİN, İsmail ÖZMEN
Suleyman Demirel University, Arts and Sciences Faculty, Department of Chemistry, Cunur, Isparta, 32260, Turkey.
selmihansahin@sdu.edu.tr

Trypsin (E.C.3.2.1.17) is an enzyme and catalyzes the hydrolysis of the arginine and lysine residues of proteins, selectively[1]. It is generally used for protein digestion in the production of food and medicine. However, the cost of the large-scale use of enzymes in solution is limiting factor for their implementation at industrial scale. Therefore, immobilization of enzymes presents an alternative approach due to the possibility of reusing enzymes and producing enzyme-free hydrolysates[1-3].

The aim of this study was to immobilize trypsin on polyvinyl alcohol (PVA)-coated magnetic nanoparticles and its characterization. Firstly, PVA-coated magnetic nanoparticle was characterized by using FTIR and TEM analyses. After that, optimization of parameters for trypsin immobilization was performed. In this study, it was also investigated that storage stability and reusability of immobilized trypsin, features that are important for practical applications. As a result, the trypsin was successfully immobilized on PVA-coated magnetic nanoparticles and after 8 usage cycles retained over 62% of its initial activity.

References:

1. Sun, J.; Yang, L.; Jiang, M.; Shi, Y.; Xu, B.; Ma, H. L., *J Chromatogr B Analyt Technol Biomed Life Sci* **2017,** 1054, 57-63.
2. Liu, J.; Liu, Y.; Jin, D.; Meng, M.; Jiang, Y.; Ni, L.; Liu, Z., *Solid State Sciences* **2019,** 89, 15-24.
3. Mao, Y.; Krischke, M.; Hengst, C.; Kulozik, U., *Food Chem* **2018,** 253, 194-202.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Identification and Correlation of miRNAs on Hypertension

Serdar KARAKURT

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY

kserdar1@yahoo.com

MicroRNAs (miRNAs) a subset of small non-coding RNA molecules downregulates gene expressions via binding to the 3′-UTR of target mRNA and play crucial roles in various pathophysiological processes including cardiovascular diseases.[1] The prevalence of hypertension has been reported from survey data collected in different industrialized countries and has ranged from 22% (Canada) to 55% (Germany) in the 1990s.[2] The aim of this study is to the identification of the interaction between aldosterone synthesis and miRNA expression and effects of miRNAs on the certain components of signaling pathways of aldosterone and its receptors. Aldosterone-induced (ALDO) rat models were generated via injecting of 0.75 µg/kg/hr of aldosterone with osmotic minipump. Quantitative amount of aldosterone was measured by Aldosterone ELISA Kit. Systolic blood pressure of rat was measured by non-invasive "Tail-cuff" method. Expression of miRNAs and mRNAs were measured via miRNA array and microarray, respectively. Following the in silico target scan-analyses miRNA candidates for aldosterone synthesis were chosen. Validation of the role of miRNAs in gene expression was accomplished via using miRNA mimic and inhibitors in human adrenal gland cells, NCI-H295r cells. Aldosterone injection was increased the blood pressure from 118±9 mmHg to 164±2 mmHg in ALDO group ($p<0.0001$). 68 miRNAs' expression and 2705 mRNAs' expression were altered. The genes and proteins related with aldosterone biosynthesis, mRNA expressions and protein expressions of CYP11B1, CYP17A1, CYP11A1, CYP21A2, AGTR1, AGTR2, NR5A2, NR3C2, MC2R HSD3B2, THOP1, ANG1, MAPK3, HSD11B1, ERK, ELK, and LNPEP were measured by qRT-PCR, western blot/ immunohistochemically, respectively. Especially, CYP11A1 (3-fold), CYP11B2 (3.3-fold) and CYP21A2 (1.6-fold) mRNA expressions were upregulated, CYP11B1 (36%) andCYP17A1 (47%) mRNA expressions were downregulated. in NCI-H295r cells. Inhibition of hsa-miR-187-3p expression with miRNA mimic was increased expression of CYP11A1 (18-fold), and CYP17A1 (4.5-fold). CYP21A2 mRNA and protein expressions were increased 12-fold after transfected with hsa-miR-128-1-5p inhibitor ($p<0.0001$). This study clarifies the molecular mechanism of aldosterone synthesis and potential marker in the regulation of hypertension

This project was supported by TUBITAK (114Z734).

References:

Bernardo, B.C.; Ooi, J.Y.; Lin, R.C.; McMullen, J.R. *Future Med Chem*. **2015**,7(13):1771-92.
Kearney, P.M.; Whelton, M.; Reynolds, K.; Whelton, P.K.; He, J. *J Hypertens*. **2004**,22(1):11-9.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Comparative Analysis of a New Microsomal Epoxide Hydrolase from *Hypsibius Dujardini*.

Velican Engin Gayır[ab], Ahmet TÜLEK[bd], Ersin KARATAŞ[bd], Barış BİNAY[ab], Elif Sibel Aslan[c]
[a] *Gebze Technical University Institue of Biotechnology Gebze Kocaeli TURKEY*
[b] *Enzim Test ve Danışmanlık Merkezi Gebze Technical University Gebze Kocaeli TURKEY*
[c] *Biruni University Department of Molecular Biology and Genetics Zeytinburnu Istanbul TURKEY*
[d] *Gebze Technical University Department of Molecular Biology and Genetics Gebze Kocaeli TURKEY*
veliengingayir@gmail.com

Epoxides are organic heterocyclic compounds containing polarized oxygen-carbon bonds. The epoxide rings are highly unstable in an aqueous medium and also exhibit nucleophilic activity which may cause irreversible toxic effects in reactions with DNA, amino acids or purines. This may lead to mutagenesis and carcinogenesis[1]. Therefore, strategies to control the epoxide concentration gain importance.

Epoxy hydrolases (EH, EC 3.3.2.9) are dimeric proteins that synthesize epoxide molecules to their respective diols. The functions of EH vary in different organisms and can be used in general to synthesize enantiomers of chiral molecules[2]. Cell receptors, hormones and enzymes are enantioselective. In addition, changes in enantiomeric forms are effective in the absorption, activation or degradation properties of drugs. For example, various anti-cancer agents and anti-HIV drugs are synthesized from substrates containing epoxide. Among the methods, bio catalysis reactions involving EH enzymes for converting epoxides to related diols are developed for this area[3].

In this study, it was aimed to elucidate a novel epoxide hydrolase protein for heterelogous expression in yeast systems via cDNA data. The specific EH gene to be used has been identified through the NCBI and UniProt databases. Studies have focused on an EH gene (*Hd-meh*) of the organism *Hypsibius dujardini*, a tardigrade of the *Eutardigrade* branch. Results of comparative analyzes (Expasy Swissmodel and NCBI BLAST), signal peptide content (Signal IP 4.1), glycosylation sites (NetNGlyc 1.0), ligand binding points and domain properties (PyMOL) were examined and epoxide hydrolase characteristics of the related gene were determined. According to result of additional BLAST and alignment applications with the protein sequences of the major studies, *Hd-meh* gene was synthesized on pPIC9K vector by GeneUniverse Biotech Inc. PCR processes were applied and band formation was observed in agarose gel analysis. In future studies, it is planned to accomplish heterelogous production, purification and kinetic calculations of enzyme in yeast cell lines.

References:

Szeliga, J., ve Dipple, A. **1998** "DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides", Chemical research in
toxicology, 11, 1, 1-11.

Saini, P., ve Sareen, D. **2017** "An overview on the enhancement of enantioselectivity and stability of microbial epoxide hydrolases", M
lecular biotechnology, 59, 2, 98-116.

Widersten, M., Gurell, A., and Lindberg, D. **2010** "Structure–function relationships of epoxide hydrolases and their potential use in b catalysis", Biochimica et Biophysica Acta (BBA)-General Subjects, 1800, 3, 316-326.

2EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Enhanced Large Scale Production of Recombinant *Staphylococcus simulans* Lysostaphin on Benchtop Bioreactor

Zeynep Efsun DUMAN[a-b*], John M. WOODLEY[c], Aişe ÜNLÜ[d-b], Hayriye ÜNAL[e], Barış BİNAY[a-b**]

[a] *Gebze Technical University, Department of Bioengineering, 41400 Gebze, Kocaeli, Turkey*

[b] *Gebze Technical University, Enzim Tanıma Merkezi, 41400 Gebze, Kocaeli, Turkey*

[c] *Technical University of Denmark, Department of Chemical and Biochemical Engineering, 2800*

[d] *Gebze Technical University, Department of Chemistry, 41400 Gebze, Kocaeli, Turkey*

[e] *Sabancı University, Nanotechnology Research and Application Center, 34956 Tuzla, Istanbul, Turkey*

Lyngby, Denmark

**Presenting Author: zeduman@gtu.edu.tr*

***Corresponding Author: binay@gtu.edu.tr*

Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) is the major cause of health-associated infections that are acquired from healthcare settings such as hospitals, dialysis centers, nursing homes, and require complex and costly treatment.[1] Therefore, the development of novel and effective strategies to combat these bacteria has been gaining significance.

Lysostaphin (EC 3.4.24.75) is a peptidoglycan hydrolase that has the ability to degrade the cell wall of almost all known staphylococcal species; in particular *S. aureus* and *S. carnosus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus, S. hominis*, *S. simulans*, *S. xylosus*, *S. hyicus* ATCC 11249 and *S. intermedius* ATCC 29663.[1-2] Although lysostaphin has significant potential on biotechnological applications such as bacteriocin, the high production costs limit its usage for healthcare settings (1 mg–134 €/ Sigma-Aldrich).[3] Regarding this issue, the scale-up studies of lysostaphin by decreasing the production cost of the enzyme on larger scales become important.

This study aims to investigate the expression of recombinant lysostaphin on a stirred tank bioreactor (STR) with different fermentation parameters such as agitation (800 rpm and 400 rpm). In parallel, the engineered auto induced For-medium consisting of different amounts of glucose and glycerol were tested. An expression cassette was constructed via transforming a pBAD vector containing *Staphylococcus simulans* lysostaphin gene into *Escherichia coli* TOP 10 competent cells. In conclusion, the tested conditions improve the protein yields up to 184.076 mg/L in a 3-L STR benchtop bioreactor. Outputs of the study will contribute to further larger-scale production of lysostaphin.

Acknowledgment: We would like to thank TÜBİTAK (project no.216M012) for financial support to this study.

References

1) Bastos M.C.F., Coutinho B.G., Coelho M.L.V., *Pharmaceuticals* **2010**, 3, 1139-1161.

2) Wu J. A., Kusuma C., Mond J. J., Kokai-Kun J. F., *Antimicrobial Agents and Chemotherapy* **2003**, 47 (11), 3407-14.

3) Szweda P., Gorczyca G., Filipkowski P., Zalewska M., Milewski S., *Preparative Biochemistry and Biotechnology* **2014**, 44:370–381.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Cloning of a novel laccase gene from *Madurella mycetomatis* and expression in *Pichia pastoris*.

Ahmet TÜLEK[ac*], Ersin KARATAŞ[ac], Barış BİNAY[bc**]

[a] *Gebze Technical University Department of Molecular Biology and Genetics Gebze Kocaeli TURKEY*
[b] *Gebze Technical University Department of Bioengineering Gebze Kocaeli TURKEY*
[c] *Enzim Test ve Danışmanlık Merkezi Gebze Technical University Gebze Kocaeli TURKEY*
**Presenting Author a.tulek@gtu.edu.tr*
** Corresponding Author binay@gtu.edu.tr*

Laccases are blue multi copper oxidases catalyzing the reduction of oxygen to water coupled with oxidation of aromatic compounds. Laccases are currently accepted as highly interesting industrial enzymes because of their useful applications for several biotechnological processes such as the detoxification of the industrial effluents from the paper, pulp and textile industries, the bioremediation of the herbicides and pesticides, the processing of the beverages (wine, fruit juice and beer), the determination of the ascorbic acid, the gelation of the sugar beet pectin, baking, being as ingredients in cosmetics and as biosensor.

Laccases often exist in gene families, and laccase isoenzymes in the same organism display diverse expression patterns and physicochemical characteristics. In order to facilitate industrial laccase applications, it is necessary to discover and evaluate a large number of laccases. However, much published work has been focused on only one or a few predominant laccases secreted under laboratory culture conditions. Heterologous expression of individual laccase genes, on the other hand, has become a valuable approach to unravel the biochemical nature and structure-function relationships of these industrially important enzymes. Heterologously expressed laccases can also be engineered for enhancement of performance (e.g., improved activity and stability, and altered catalytic properties) with directed evolution or site-directed mutagenesis.

In the present work, we report genetic and biochemical characterization of a novel laccase, designated as *MmLac*, from *Madurella mycetomatis*. There are no any other laccases have been purified and characterized from *Madurella mycetomatis*. This report would further our understanding of *Madurella mycetomatis* laccase and investigates its biochemical properties. For this purpose, we isolated *MmLac* gene from *Madurella mycetomatis* and transformed it into *Escherichia coli TOP10* cells via pPICZαA vector. After plasmid isolation *MmLac* containing pPICZαA expressed in *Pichia pastoris*. The enzyme has a molecular mass of about 62 kDa. The K_m and k_{cat} values of *Mm*Lac toward ABTS were 38,43 µM and 215.84 s^{-1}, respectively.

Acknowledgement: We would like to thank the TUBITAK group (project number 119Z580) who provided financial support for this work.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Synthesis of Copper Loaded Magnetic Iminodiacetate Nanoparticles and Its Use on The Immobilization of Cellulase Enzyme

Elif Özyılmaz[a], Özge Çağlar[b], Ahmed İ. Alhilali[b]
[a] *Department of Biochemistry, Selcuk University, 42075 Konya, Turkey*
[b] *Department of Chemistry, Selcuk University, 42075 Konya, Turkey*
e-posta: *ozgecaglar07@gmail.com*

Magnetic nanoparticles are generally of low toxicity and that they can easily be separated from reaction mixtures by means of an external magnet has increased the interest in the use of magnetic particles as support material in enzyme immobilization in recent years.[1]

Cellulase is important hydrolytic enzyme used in many industries including food industry, textile industry, paper industry. As high specifity of cellulase enzymes, non-toxic, biodegradability and moderate pH, temperature, pressure and are more advantageous than inorganic catalysts, but limiting its use in industry due to production costs.[2] Enzyme immobilization is an important method to ensure the stability and reusability of enzymes.[3]

In this study, iminodiacetic acid and 3-(triethoxysilyl) propyl isocyanate were activated and used for in cellulase immobilization. For this purpose, Fe_3O_4 was synthesized according to the method mentioned in the literature and after synthesis it was magnetized by magnetic nanoparticles.[4] The resulting compound was converted into Cu^{+2} charged magnetic nanoparticles by reacting with the Cu^{+2} ions. The synthesized compound was used for cellulase immobilization under suitable conditions after being characterized by spectroscopic techniques. The activity of immobilized enzymes, optimum pH and temperature conditions, thermal stability, reusability and kinetic parameters was investigated.

References:

Cai, Z.; Wei, Y.; Wu, M.; Guo, Y.; Xie, Y.; Tao, R.; Li, R.; Wang, P.; Ma, A.; Zhang, H. *ACS Sustainable Chem. Eng.* **2019**, 7, 6685−6695.
Han, J.; Wan, J.; Wang, Y.; Wang, L.; Li, C.; Mao, Y.; Ni, L. *ACS Sustainable Chem. Eng*. **2018**, 6, 7779−7788.
Ozyilmaz, E.; Bayrakci, M., Yilmaz, M. *Bioorg. Chem.* **2016,** *65*, 1–8.
Yilmaz, E. ; Sezgin, M.; Yilmaz, M. *Biocatal. Biotransform*. **2009**, *27*, 360-366.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Magnetic stimulated cryogels to enhance stem cell differentiation

Sedat Odabas[1], Atakan Tevlek[2], Berkay Erenay[3] Halil Murat Aydın[2] , Batur Ercan[4] , Bora Garipcan[3] , Atiye Seda Yar Saglam[5]

[1] *Ankara University, Faculty of Science, Department of Chemistry, Ankara, TURKEY*
[2] *Bioengineering Division, Institute of Science and Engineering, Hacettepe University, Ankara, TURKEY*
[3] *Bogazici University, Institute of Biomedical Engineering, Istanbul TURKEY*
[4] *Metallurgical and Materials Engineering, Middle East Technical University, Ankara, TURKEY*
[5] *Department of Medical Biology and Genetics, Faculty of Medicine, Gazi University, Besevler, Ankara, TURKEY*
odabas@ankara.edu.tr

Magnetic nanoparticles are used in several technical fields like electronics, catalysis technology as well as fundamental medicine and biomedical. Here we propose to use magnetic nanoparticles as an efficient way for mechanotransductive effect on cells to promote growth or differentiation. Briefly, Super-paramagnetic particles were synthesized by co-precipitation method. Size, size distributions and magnetic characteristic of the synthesized particles were analyses with TEM and VSM. Magnetic cryogels were prepared by a slight modification of previous reports[1,2] and cross-linked using NHS/EDC carbodiimide chemistry. Chemical and structural characterizations were performed with X-RD, VSM and SEM. Mesenchymal stem cells were seeded on these magnetic cryogels. Cells were cultured in osteogenic and chondrogenic media up to 28days under 3 conditions: (I) Non-magnetic field environment (II) 1000 Gauss static magnetic field (III) 4000 Gauss static magnetic field. Stem cell differentiation behavior changes through Osteogenic and Chondrogenic ways were examined by several methods such as: Proliferation and Doubling time calculations, Morphological observations via SEM and Confocal Microscopy, gene expression profiling with Real Time PCR and quantitative histochemical staining. The metabolic activity changes and the differentiation behaviour of the cells through to both bone and cartilage under direct magnetic field effect and at different magnetic field strength was promising, as they promote cell proliferation and growth as well as differentiation with the ability to generate proper physical stimuli throughout the cells.

References

1) Inci, I., Odabas, S., et al. Gelatin-hydroxyapatite cryogels with bone morphogenetic protein-2 and transforming growth factor beta-1 for calvarial defects. *J. Biomat. Tissue Eng.*, **2014** *4*(8), 624-631.
2) Odabas, S. (2016). Collagen–carboxymethyl cellulose–tricalcium phosphate multi-lamellar cryogels for tissue engineering applications: Production and characterization. *J Bioact Compat Polym*, *31*(4), 411-422.

Acknowledgements: This study has been supported by The Scientific and Technological Research Council of Turkey (TUBİTAK)- 3501-Career Development Programme (Grant No: 115M522).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Phenolic Component and Antioxidant Activity of Apple Pomace: Effect of Time and Extraction Methods

Sercan OZBEK YAZICI[a], Ismail OZMEN[b]

[a]Faculty of Health Sciences, Department of Nutrition and Dietetics, Burdur Mehmet Akif Ersoy University, Istiklal Yerleskesi, Burdur, 15100, Turkey

[b]Art and Science Faculty, Department of Chemistry, Suleyman Demirel University, Cunur, Isparta, 32260, Turkey.

sozbekyazici@hotmail.com

Industrial processing of agricultural products for the production of food results in significant amounts of byproducts. Apples can be consumed as fresh or processed into various products. As a result of processing, pomace is generated and is rich in bioactive compounds.

Apple pomace is a promising source of phenolic compounds which possess significant antioxidant capacities. In this study, the effect of three different methods on the extraction of phenolic compounds from apple pomace was determined in terms of total phenolic content (TPC) , total flavonoid content (TFC) and antioxidant activity (DPPH radical scavenging activity (IC_{50})). Phenolic compounds were extracted from peach pomace using 70% ethanol (containing 1% HCl) by maceration method (ME) (30, 60, 180 min); ultrasound-assisted extraction (UAE) (10, 20 and 30 min); microwave assisted extraction (MAE) (30, 90 and 180 seconds).

In the maceration extraction method, TPC and TFC were determined as 1280.1 ± 15.1 µg GAE/g FW and 344.1±2.6 µg QE/g FW at 180 min, respectively. In the ultrasound-assisted extraction, TPC and TFC were measured as 1010.4±3.3 µg GAE/g FW and 256.7±1.7 µg QE/g FW at 30 min, respectively. In microwave assisted extraction, TPC and TFC were determined that 1603± 11.9 µg GAE/g FW and 288.9 ± 9.1 µg QE/g FW at 90 s. The highest DPPH IC_{50} value was 1.47± 5.2mg/ml for ME at 180min, 1.61± 5.2 mg/ml for UAE at 30min, and 0.91± 3.1 mg/ml for MAE at 90s.

In the study, it can be suggested that MAE is the most effective method for the extraction of total phenolic compounds (except for total flavonoids) from apple pomace. Also, the ultrasound assisted extraction method is better in comparison to the maceration method in terms of obtaining the same amount of material in a shorter time.

Keywords: Apple pomace, Phenolic compounds, Microwave assisted extraction, Ultrasound assisted extraction, Maceration extraction

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Determination of Antiradical and Antioxidant Properties of *Asplenium scolopendrium*

Pınar Kalın

Department of Chemistry, Faculty of Science, Atatürk University, 25240 Erzurum, Turkey

pannakaya@atauni.com

Asplenium scolopendrium (A. scolopendrium) is a perennial fern that can grow to 20-50 cm. Turkey's coastal areas in the forest; fir, beech and rocky forests; They can develop naturally in damp, shady, stony and rocky mountain areas.[1] Phenolic substances have been identified in foliage, fruits, seeds, vegetables, shells, flowers. Such compounds have begun to be preferred for medical purposes as well as being part of the natural way of feeding. In previous times, people applied to herbal remedies to solve their health problems. Today, scientific studies have increased, especially as alternative medicine has shown.[2] In this study, it was aimed to determine the antioxidant capacity by using ethanol extracts of *A. scolopendrium*. For this purpose, antioxidant and antiradical properties were determined by different bioanalytical methods *in vitro* such as determination of iron and copper reduction capacities, ABTS and DPPH radical scavenging activities, total phenolic and flavonoid compound amounts by using ethanol extracts of *A. scolopendrium*. An increase in concentration was observed in the reduction methods. Capacities to reduce ferric ions (Fe^{3+}) at a concentration of 20 µg/mL; BHA (1.785), BHT(0.991), Trolox(1.439), α-Tocopherol(1.021), *A. scolopendrium*(0.286). It has been detected that the methods have high antioxidant capacity.

References:

Testo, W. L., Watkins Jr., J. E. *Am. J. Bot.* **2013,** 100, 2261-2270.
Topal, M. *Anadolu J. Agr. Sci.* **2018,** 33, 232-236.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

One-Step Isolation and Biochemical Characterization of The Peroxidase Enzyme from Jerusalem Artichoke (*Helianthus Tuberosus* L.)

Ramazan KALIN

Department of Basic Science, Faculty of Sciences, Erzurum Technical University, 25240-Erzurum, Turkey

ramazan.kalin@erzurum.edu.tr

Enzymes are used in a variety of fields including medicine, pharmaceuticals, food processing, cosmetics, chemical industry, agriculture, biodiesel production, textile, detergent, animal feed production and energy.[1] Peroxidases catalyze the oxidation of both organic and inorganic substrates in the presence of H_2O_2. Peroxidases are one of the enzymes that have the potential to reduce environmental pollution. It is especially used in the treatment of waste water.[2]

In this study; the peroxidase (POD) enzyme from jerusalem artichoke (*Helianthus tuberosus* L.) purified for the first time in a single step by the affinity column prepared from the 4-aminobenzohydrazide molecule which is one of the peroxidase inhibitors.[3] Enzyme activity measured spectrophotometrically at 470 nm. The purity of purified enzymes were determined by SDS-PAGE. The purification results of the peroxidase enzyme purified from jerusalem artichoke were found to be 87.17 times with a yield of 13.8%. Optimum parameters such as pH, ionic strength, temperature were determined for purified jerusalem artichoke POD. Also, K_m and V_{max} values of H_2O_2 and guaiacol substrates were calculated for POD. Finally, the inhibition values of the 4-aminobenzohydrazide molecule used as a ligand on purified POD were determined. IC_{50} values were found to be 1.469 mM.

References:

Jemli, S.; Ayadi-Zouari, D.; Hlima, H. B.; Bejar, S. *Critical Rev. Biotechnol.* **2016,** 36, 246.
Ong, S. T.; Keng, P. S.; Lee, W. N.; Ha, S. T.; Hung, W. T. *Water*. **2011,** 3(1), 157.
Kalin, R.; Atasever, A.; Ozdemir, H. *Food Chem.* **2014**, *150*, 335.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Inhibition Effects of Some Flavonoids on Beta-Lactamase Enzyme and Determination of Synergistic Antimicrobial Activities with Beta-Lactam Antibiotics.

Aykut Öztekin[a], Veysel Çomaklı[b], Kenan Karagöz[c], Şevki Adem[d],

[a]*Agri Ibrahim Cecen University, Vocational School of Health Services, Department of Medical Services and Techniques, , Agri, Turkey*
[b]*Agri Ibrahim Cecen University, School of Health, Department of Nutrition and Dietetics, Agri, Turkey*
[c]*Agri Ibrahim Cecen University, Faculty of Science Literature, Molecular Biology and Genetics Department, Agri, Turkey*
[d]*Cankiri Karatekin Universty, Faculty of Science Literature, Chemistry Department, Cankiri, Turkey*

kkaragoz@agri.edu.tr

Flavonoids are natural antioxidants, which are usually in polyphenolic form. β-lactamase enzymes are the main factors of bacterial resistance to antibiotics and they significantly reduce the effect of β-lactam antibiotics. For this reason, studies on inhibition of β-lactamase enzyme is important. New inhibitors that may be present will have potential to increase the effectiveness of β-lactam antibiotics. In this study, Inhibition effect of 6-hydroxy flavone, 6-fluoro flavone, 3,5,7-Trihydroxy-2- (3,4,5-trihydroxyphenyl) -4H -chromen-4-one, 7-Hydroxy-4'-nitroisoflavone, 5,7-Dihydroxy-2- (3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one molecules on beta-lactamase enzyme. IC_{50} values were found to vary between 1.01 µM and 173.3 µM. All the molecules were tested against *Klebsiella pneumoniae* ATCC 700603 and *Ecsherichia coli* ATCC 35128, which resistant to Penicillin G. According to our results, 1 mg / ml^{-1} Penicllin G alone didn't hedge growth of the bacteria when compared the control applications. However, when the same dose Penicillin G combined with the molecules, Especially, 6-fluoroflavone and 7-Hydroxy-4'-nitroisoflavone molecules were found to be highly effective against *K. pneumoniae* ATCC 700603 and *E. coli* 35128.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Fast and Reliable New Methods in Oncologic Early Diagnosis: BEAMingPCR / Digital PCR and CellSearh Assay

Serkan ŞEN

Afyonkarahisar Health Sciences University, Ataturk Vocational School of Health Services

serkansen@aku.edu.tr

The BEAMing technique is based on clonal amplifications of single DNA molecules on mil-lions of primer -coated magnetic beads within 3- to 5-micron diameter in size oil emulsions followed by flow cytometric analysis of the DNA coated beads. Using this technique, the gender of a 4-week-pregnant women could be determined by fetal DNA detection in maternal serum sample. In this study, the researchers used a digital PCR technology called BEAMing (beads, emulsion, amplification, magnetics) to determine male circulating fetal DNA (cfDNA) in maternal plasma targeting the sequences of the amelogeninY (AMELY) gene specific to the Y chromosome. In this study, the known amounts of DNA were predicted with high accuracy to reliably detect fetal DNA in maternal plasma of 4-week pregnant women using the BEAMing technique. In another study, Diehl et. al. applied a very sensitive approach to quantify tumor DNA (ctDNA) in 162 plasma samples from 18 subjects who have received multimodality treatment for colorectal cancer. They demonstrated that ctDNA quantifications can be used to reliably monitor tumor dynamics in cancer patients undergoing surgery or chemotherapy, and suggested that this personalized genetic approach can generally be applied to individuals with other types of cancer. In this study, ctDNA was detected in all subjects preoperatively, and serial blood sampling revealed oscillations associated with the size of surgical resection at the ctDNA level. In the subjects with pre-operatively detectable ctDNA, the disease has reccurred generally within 1 year. It was concluded that ctDNA can be more reliable and precise marker than the existing standard biomarker (CEA) in this subject cohort. In this respect, ctDNA quantifications by BEAMing PCR show that ctDNA levels reflect total systemic tumor burden, and that ctDNA levels decrease after full surgery and generally increase as new lesions appear on radiological examination. However, whether ctDNA levels are exactly proportional with systemic tumor burden cannot be now determined exactly because of the lack of independent way to quantify the systemic burden. When it is quantified by radiography, dead tumor cells may be not noticed, however; when ctDNA is quantified, only information on tumoral DNA in the circulation is obtained but a numeric data on tumor burden at tumor sites cannot be obtained yet.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In vitro studies of Doxorubicin and Curcumin Loaded Magnetic Nanoparticles in Lung Cancer Cell Line

Güliz Ak [a,b] *Tuğba Karakayalı* [a], *Ayşe Nur Cin*[a], *Buket Özel* [b,c] *Şenay Hamarat Şanlıer* [a,b]

[a] *Ege University Faculty of Science Biochemistry Department, Izmir, Turkey*
[b] *Ege University Center for Drug Research, Development and Pharmacokinetic Applications (ARGEFAR), Izmir, Turkey*
[c] *Ege University Faculty of Medicine Department of Medical Biology, Izmir, Turkey*
tugbakrkyl@gmail.com

Abstract

This study was aimed to investigation of cytotoxic effects of Doxorubicin (Dox) and Curcumin (Cur) loaded magnetic nanoparticles (NP) against A549-luc-C8 lung cancer cell line. The combination of Dox and Cur is used for reduce the side effects and toxicity while maintaining the therapeutic properties of drugs through dual therapy. MTT assay was performed to test cytotoxicity effect in A549-luc-C8 cell line. IC50 doses of Dox was 0.126 µg/mL and Cur was 24.1 µg/mL on A549-luc-C8 cell line. IC50 values of Dox-loaded NP and Cur-loaded NP were obtained as 251.2 µg/mL and 102.7 µg/mL. Dox-Cur-NP were more effective than single drug-containing nanoparticles. The combination index (CI) was calculated to investigate combination treatment of Dox and Cur by Calcusyn Programme. Synergism and antagonism are based on the median-effect principle and the CI–Isobologram Theorem. Combination of Dox and Cur in magnetic nanoparticles showed synergistic effect and Combination Index value of Dox-Cur-NPs was under 1. Results showed that the prepared NPs had synergistic antitumor activity for non-small cell lung cancer.

Refrences

1) Hanahan, D.; Weinberg, R. A. The Hallmarks of Cancer. Cell 2000, 100 (1), 57–70. https://doi.org/10.1007/s00262-010-0968-0.
2) Sarkar, S.; Horn, G.; Moulton, K.; Oza, A.; Byler, S.; Kokolus, S.; Longacre, M. Cancer Development, Progression, and Therapy: An Epigenetic Overview. Int. J. Mol. Sci. 2013, 14 (10), 21087–21113. https://doi.org/10.3390/ijms141021087.
3) Greco F, Vicent MJ. Combination therapy: Opportunities and challenges for polymer-drug conjugates as anticancer nanomedicines. Adv Drug Deliv Rev. 2009.
4) So HN, Hye YN, Jae RJ, In SB, Park JS. Curcumin-loaded PLGA nanoparticles coating onto metal stent by electrophoretic deposition techniques. Bull Korean Chem Soc. 2007;28:397–402.
5) Chou TC. Drug combination studies and their synergy quantification using the chou-talalay method. Cancer Res. 2010;70:440–6.
6) Peters GJ, Van Der Wilt CL, Van Moorsel CJA, Kroep JR, Bergman AM, Ackland SP. Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol Ther. 2000.

Innovative Approaches In Protein Purification to Drive Efficiency

Stephan Poetsch

Abstract:

With the anticipated growth in diversity of molecules under discovery and research, and the ever-increasing pressure to improve productivity, flexibility, and cost efficiency, there will be a relentless focus on implementing different research tools and innovative approaches to achieve improved research outcomes. This speech will discuss protein purification tools and approaches to address current challenges in research and drive efficiency while providing information about the trends in academia and industry. Speaker will also review the newest chromatography media with improved binding capacity to resolve bottlenecks in production and exceptional alkaline stability for efficient cleaning and sanitization.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Identification of the importin α subtype that is responsible for the import of HNF1A transcription factor into the nucleus and analysis of nuclear localization signal on HNF1A protein

Fareed Mohammed Ali Fareed[a], Barbaros Nalbantoğlu[a], Özlem Yalçın Çapan[b*]

[a]*Department of Chemistry, Yıldız Technical University, Esenler, İstanbul, Turkey*
[b]*Department of Molecular Biology and Genetics, İstanbul Arel University, Büyükçekmece, İstanbul, Turkey*

*Presenting author: ozlemyalcin@arel.edu.tr

Maturity-onset diabetes of the young (MODY) is a monogenic subtype of diabetes mellitus. The most common type is MODY3 that is associated with mutations in transcription factor gene, HNF1A. In previous studies R271W and S345Y mutant HNF1A were found to be defective in nuclear localization in MODY patients. Importin α receptor family are responsible for translocating proteins into the nucleus. Importin proteins recognize and bind to nuclear localization signal (NLS) motifs on cargo proteins. Although classic NLS motifs are known to consist of basic amino acids, non-classical NLS motifs were also reported. One of the purposes of the study is to identify the importin α subgroup which is responsible for the transport of HNF1A protein into the nucleus. Second purpose is to analyze the interaction between pre-determined importin α subgroup and mutant HNF1A proteins (R271W and S345Y). R271 is located in a classical NLS while S345 in a non-classical NLS region of HNF1A.

In this study, the interactions of wild type and mutant HNF1A proteins with the three candidate mouse homolog importin α proteins (Kpna2, 4 and 6) were evaluated by co-immunoprecipitation (Co-IP) technique. To confirm this interaction, specific importin α protein was knockdown by siRNA in mouse pancreatic cells (MIN6) and the location of HNF1A was analyzed by immunochemical staining techniques. Co-IP studies with wild type HNF1A and importin α subgroups revealed that strongest interaction was with mouse Kpna6 protein. When Kpna6 is silenced by siRNA treatment, there is 10% reduction in the nuclear localization of HNF1A protein. Also Co-IP studies with mutant HNF1A proteins (R271W and S345Y) and Kpna6 showed there is a reduced interaction compared to the wild type confirming the role of these regions as NLS.

As a conclusion, the results of the study clarify the molecular pathway of nuclear import and nuclear localization signals of HNF1A protein and molecular pathogenesis of HNF1A mutations in MODY3 patients.

This study was supported by TUBİTAK (Project no: 116Z564)

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Microbiota-derived Postbiotic Mediators: A Novel Formulation For Oral Health Management

Fadime Kiran, Hazal Kibar, Başar Karaca
Ankara University, Faculty of Science, Department of Biology, Ankara, TURKEY
fkiran@science.ankara.edu.tr

Microbiota represents an ensemble of microorganisms that live in or on the human body. Microbiota-derived beneficial bacteria can effect on overall human health via improving the micro-ecological balance of the host. Recently, attention on the potential health benefits of microbiota has been focused on their production of soluble factors, defined as "postbiotic mediators" which are able to exert beneficial properties against pathogen growth, pathogen-induced inflammation and related alteration of cytokine release. The aim of this study was to evaluate whether postbiotic mediators harvested from microbiota originated commensal bacteria protect oral health. For this purpose, anti-microbial, anti-biofilm and anti-quorum sensing activity of the microbiota-derived postbiotic mediators were tested against *Streptococcus mutans* ATCC 25175, which is one of the most important pathogen for oral health. In addition, antioxidant activity was also assessed. Our results showed that postbiotic mediators have the potential for inhibition of *S. mutans* growth. Biofilm formation of this pathogen, measured by crystal violet assay following the 24h co-incubation by sub-MIC value of lyophilized postbiotic mediators, was also inhibited with the reduction rates between 70% and 90%. Moreover, the antioxidant activity of postbiotic mediators was demonstrated through DPPH radical scavenging activity with the range from 60% to 85%. The foregoing data have formed a basis for future clinical studies to evaluate the beneficial oral health effects of microbiota-derived postbiotic mediators which can be used as an alternative instead of antibiotics to decrease the chance of dental plaque formations as well as dental caries by reducing the count of S. *mutans*.

POSTER

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Screening of secondary metabolites, total phenolics and flavonoid contents, characterization of phenolics compounds, and in vitro evaluation of biological activities of *Thymus fontanesii* Boiss et Reut extracts.

Ahmed Nouasri[1], Soumeya Krimat[1], Dahmane Dahmane[1], Yacine Badja[3], Mohamed-Amine Ferhat[1], Abdel Krim Kameli[2].

1 Laboratory of bioactive products and biomass valorization research. ENS kouba, Address: BP92, vieux kouba Alger, Algeria.

2 Department of natural sciences, ENS kouba, Address: BP92, vieux kouba Alger, Algeria.

3 King Saud University | KKUH (Arabie Saoudite) · Department of Chemistry.

Corresponding Author Email : a_nouasridz2001@yahoo.fr

Abstract

The hydro-methanolic extract and its fractions of aerial parts of *Thymus fontanesii Boiss et Reut* were investigated for their phytochemical screening, total phenolic and flavonoid contents, Attempt to identify phenolics compounds4, antioxidant, antimicrobial activities, Common standard tests were done to put in evidence the presence of chemical families. Phytochemical screening revealed the presence of the flavonoids, coumarins, tannins, the anthraquinones derivatives and combined O- and C-heterosides, and the absence of alkaloids. The characterization of the crude hydro-methanolic extract by HPLC UV-DAD, has revealed the presence of: vanillin, salicylic acid, and other acids. Total phenolics and flavonoids contents of the extracts varied between 14.56-237.6 mg GAE/g extract and 1.63– 2.27 mg QE/g extract, respectively where the ethyl acetate and diethyl ether extract have the highest values. In-vitro antioxidant activities were performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power and β-carotene–linoleic acid tests. and compared with standards compounds such as (BHT), ascorbic acid and α- tocopherol. The results indicated that for Antiradical test the diethyl ether fraction exhibited stronger scavenging activity, for the FRAP test methanolic extract were more active than other fractions, at least the chloroform fraction was the stronger protector of β-carotene–linoleic acid system. Antimicrobial activity was examined against 6 bacteria and 4 fungi. *Listeria monocytogenes, Pseudomonas aeruginosa and E. coli Aspergillus flavus, and Penicillium expansum* were not inhibited by plants extracts. Diethyl ether and chloroformed fractions was generally more active than others. Considering these results, *T. fontanesii,* can be used as a source of novel antimicrobial and antioxidant compounds.

Keywords: *T. fontanesii,* characterization of phenolics compounds, biological activities.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

New Dithiolopyrrolone Antibiotic Induced by Adding Cinnamic Acid to the Culture Medium of *Saccharothrix Algeriensis* NRRLB-24137

Merrouche Rabiaa[a], Sabaou Nasserdine[a], Yannick Coppel[b], Mathieu Florence [c] and Zitouni Abdelghani[a].

[a], *Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale supérieure de Kouba, Alger, Algérie.*
[b], *Laboratoire de Chimie de Coordination (LCC), CNRS, 205 route de Narbonne, 31077 Toulouse, France.*
[c], *Université de Toulouse, Laboratoire de Génie Chimique UMR 5503 (CNRS/INPT/UPS), INP de Toulouse/ENSAT, 1 avenue de l'Agrobiopôle, Castanet-Tolosan Cedex, France.*

Email address
merrabiaa74@yahoo.fr

Abstract

Saccharothrix algeriensis NRRL B-24137 (=DSM 44581) is an actinomycete which was isolated from Algerian Saharan soil. This strain of actinomycete produces bioactive compounds belonging to the dithiolopyrrolone class of antibiotics.[1, 2]

Dithiolopyrrolone antibiotics has a broad-spectrum activity against a variety of microorganisms including gram-positive and gram-negative bacteria, yeasts, ameboid parasites and phytopathogenic fungi. This class of antibiotics generated new interest after the discovery of their anticancer and antitumor properties.[3]

In this study, a new antibiotic was purified from the fermentation broth of *Saccharothrix algeriensis* NRRL B-24137 and characterized as dithiolopyrrolone derivative. This natural antibiotic was induced by the addition of cinnamic acid, as precursor, at a concentration of 5 mM to the semi-synthetic medium (SSM). The analysis of the induced antibiotic was carried out by HPLC system equipped with a C18 reverse phase column. The chemical structure of this antibiotic was determined by ^{1}H- and ^{13}C-nuclear magnetic resonance, mass and UV-visible data. This dithiolopyrrolone antibiotic was characterized as benzoyl-pyrrothine. The minimum inhibitory concentrations of this new induced antibiotic was determined.

References

Merrouche, R.; Bouras, N.; Coppel, Y.; Mathieu, F.; Monge, M. C.; Sabaou, N.; Lebrihi, A. *J Nat Prod*. **2010**, 73, 1164–1166.

Merrouche, R.; Bouras, N.; Coppel, Y.; Mathieu, F.; Sabaou, N.; Lebrihi, A. *FEMS Microbiol. Lett*. **2011**, 318, 41–46.

Oliva, B.; O'Neill, A.; Wilson, J. M.; O'Hanlon, P. J.; Chopra, I. *Antimicrob. Agents Chemother.* **2001**, 45, 532–539.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Phytochemical analysis and evaluation of antioxidant activity of Teucrium flavum extracts.

R. Senani [a], S. Krimat[b], M. Boudjniba[a]

[a]*Laboratory of ethnobotany and natural substances. ENS kouba, Alger-Algeria*

[b]*Laboratory of bioactive products and biomass valorization research.ENS kouba, Alger-Algeria*

Email: r_senani@yahoo.fr

Abstract: Total phenolic content**,** flavonoid concentration and antioxidant activity were determined by spectrophotometric methods in Teucrium flavum extracts. Water methanolic, ethyl acetate and aqueous extract were analyzed. The concentration of total phenolic compounds was determined by Folin-Ciocalteu reagent and the obtained values ranged from 157.45 to 189.38 mg EAG/g of extract. The concentrations of flavonoids in plant extracts of T. flavum ranged from 3.20 to 5.24 mg EQ/g of extract. The evaluation of antioxidant capacity by the method of free radical scavenging test showed that all of the extracts have a very good reductive activity, especially for ethyl acetate extract which presented a percentage of inhibition equal to 90% with an IC50 estimated to 12.76 ug/ml. On the other hand, the FRAP test and β-carotene bleaching method revealed that the aqueous extract has the best reducing power than those of the other extracts, but it remains relatively low compared to the ascorbic acid used as positive control. The obtained values show that some extracts of T. flavum are very rich source of phenolic compounds with strong antioxidant activity. The extract of T. flavum, could be considered as a source of potential antioxidants and will promote the reasonable usage of these plants in food technology and processing as well as for medical use.

Keywords: antioxidant activity. Total phenolic content, Total flavonoid content. DPPH

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Total phenolics, flavonoids contents and antioxidant properties of different extracts of *cymbopogon citratus* leaves from algeria

Lakache Zineb, Tigrine Chafia and Kameli Abdelkarim.

Laboratoire d'ethnobotanique et substances naturelles, Ecole Normale Supérieure, Kouba, Alger, Algérie

lakache.zineb@yahoo.fr

Abstract

Cymbopogon citratus, commonly known as lemon grass, mainly used in food and Algerian traditional folk medicine for the treatment of nervous and gastrointestinal disturbances. The aim of the present study is to investigate the antioxidant activities of different extracts (ethanol extract, infusion and decoction) obtained from Algerian lemon grass leaves using different tests such as DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and β-carotene assays. The total phenolic and flavonoid contents were determined using Folin-Ciocalteu and aluminium chloride assays respectively. The quantitative estimation of total polyphenols and flavonoïds showed their existence in all extracts, where ethanol extract of *Cymbopogon citrates* is the richest in phenolic compounds (4.40 ± 0.52 mg equivalent of gallic acid/g of extract) compared with infusion and decoction (1.32 ± 0.09 and 0.52± 0.03 mg equivalent of gallic acid/g of extract respectively).The evaluation of the antioxidant activity by DPPH showed that the studied extracts have a very good antioxidant activity, especially the ethanol extract of *Cymbopogon citrates* with an IC50 of 10.6 µg/ml, followed by infusion and decoction extracts with an IC50 value of 13.3 and 15.1 µg/ ml, respectively. In β-carotene bleaching test, the oxidation of β-carotene was effectively inhibited by different extracts of *C.citrates*, especially the ethanol extract. As a conclusion, the results of the present study indicate that the aerial part extracts of *Cymbopogon citrates* is a good source of natural antioxidant constituents.

Keywords: Cymbopogon citrates, total polyphenols, flavonoïds, antioxidant activities.

2.EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Effect Of Enzymatic Hydrolysis Of Milk Proteins On The Hypoallergenicity Of Dairy Products

Rugiya Mustafayeva, Naila Abdullayeva, Natavan Bakhshaliyeva

Baku State University, AZ1148, Z.Khalilov str.23, Baku, Azerbaijan

natavanscience@gmail.com

By enzymatic hydrolysis of the protein component of milk, products with low allergenic potential can be obtained. During the hydrolysis of casein, the peptides formed under the action of intracellular peptidases are split into short-chain peptides and amino acids. As a result, during the fermentation of casein, some lactic acid bacteria produce biologically active peptides that are relevant in the development of dairy products.

Allergenicity of dairy products can be reduced under the action of various technological methods. To do this, the molecular allergenic potential of proteins changes, the region of antigenic epitopes is destroyed or new epitopes are formed. Enzymatic hydrolysis is one of the ways to reduce the allergenicity of dairy products.

The mechanism of enzymatic catalysis consists of three consecutive reactions. First, the Michaelis complex is formed from protein and enzyme. The second reaction is peptide bond cleavage and release of a single peptide. At the last stage, the resulting peptide is separated from the enzyme after a nucleophilic attack, which is carried out by a water molecule.

Casein is considered the main source of protein for the production of hypoallergenic protein hydrolysates. But the main disadvantage of proteolysis of casein is an unpleasant taste and smell. Proteolysis of casein conductors by the method of Mouecoucou and co-authors. Casein hydrolysis was carried out in 2 stages: protease hydrolysis by strains of lactic acid bacteria and pepsin hydrolysis. Pepsin hydrolysis was carried out for 1 hour at pH 2.0 and a temperature of 37 ° C. As a result, preliminary hydrolysis of caseins by proteases of a strain of lactic acid bacteria improved their digestion with pepsin. Thus, to reduce the allergenicity of proteins, a method of enzymatic hydrolysis was used, which is predominant in the production of dairy products.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

ENVIRONEMENTAL EFFECT ON ANTIOXIDANT PROPERTIES OF *PHLOMIS BOVEI DE NOE SUBSP BOVEI* .

Mouas Y , Tigrine C, Tounsi H, Forar EL Aidi R and Kameli A .

1: Laboratory of Ethnobotany and Natural Substances, Ecole Supérieure Normale (ENS), Vieux Kouba .Alger

mouasyamina @gmail .com

Abstract

In this study, four hydroalcoholic extracts are prepared at from the leaves of the plant *Phlomis bovei Dé Noé subsp bovei the same phenological stage (flowering).* The quantification of polyphenols and flavonoids total was achieved using a colorimetric method. The antioxidant activity of the prepared extracts was evaluated by 3 different tests: DPPH free radical scavenging test. , Reducing Power Test (FRAP) and B_carotene bleaching test. The comparative study between these samples was based on 2 criteria: Altitude and environmental criteria (pollution), these are Beni Ali (727 m) and Chrea (1379m). For the same altitude two variants were distinguished: one polluted and the other not. The antioxidant evaluation by DPPH showed that the extracts studied from Beni Ali showed a high activity compared to the second region. Concerning the bleaching test of B-carotene, extracts from both regions exerted a significant antioxidant effect. On the other hand, the reducing power method revealed that the polluted Beni Ali sample has the best reducing power compared to that of Chréa. The quantification of polyphenols and total flavonoids has shown that the content of natural Beni Ali extract is greater in comparison with Chréa.

Keywords: *Phlomis bovei* , altitude, pollution, antioxidant activity, phenological stages.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Green Extraction and Chromatography of Carotenoid from Tomato

[a] Mohamed Amine Ferhat , [b]Mohamed Nadjib Boukhatem

[a]*Laboratoire de Recherche sur les Produits Bioactifs et la Valorisation de la Biomasse LPBVB, Ecole Normale Supérieure Vieux Kouba ENS BP 92 Kouba- Alger, Algeria.*

[b]*Laboratoire Eco-Physiologie Végétale, Département des Sciences Naturelles, Ecole Normale Supérieure de Kouba, Alger, Algeria*

Ferhatamine100@yahoo.fr

Lycopene is the main carotenoid in tomato. Used a long time like simple food dye, it is today the subject of many studies as for its positive effects on health. To avoid this shortcoming, a useful and green method for the extraction of lycopene with a new procedure using d-limonene (bio-solvent) a substitute for petroleum solvent has been proposed. d-limonene from the orange peel was extracted through a steam distillation procedure followed by a deterpenation process and combining this achievement by using it as a solvent for extracting lycopene from tomato fruit as a substitute of dichloromethane. Lycopene extracted from tomato fruit were compared with both conventional petroleum and bio-solvent in terms of qualitative and quantitative determination. No significant difference was obtained between each extracts allowing us to conclude that the proposed method is effective and valuable. The proposed approach using a green solvent to perform extraction is useful and can be considered as a nice alternative to conventional petroleum solvent where toxicity for both operator and environment is reduced.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Essential Oil Composition and Antioxidant Properties of The Aerial Parts of *Pituranthos Scoparius* (Coss and Dur) Schinz (Apiaceae) From Hoggar, Southern Algeria

Ksouri Aicha[1,2]*; Krimat Soumia[1]; Dahmane Dahmane[1]; Belkebir Aicha[2].

[1]*Laboratory of Bioactive Products and Biomass Valorization Research, École Normale Supérieure (ENS-Kouba).*

[2]*Laboratory of Plant Physiology, Faculty of Biology Science, University of Science and Technology Houari Boumedienne (USTHB), Algiers, Algeria*

**For correspondence: Email: ksouri_aicha@hotmail.com*

Abstract

To determine the chemical composition as well as in vitro antioxidant activity of the *Pituranthos scoparius* essential oil. The chemical composition of a hydro-distilled essential oil of *P.scoparius* was analyzed by GC and GC/MS systems. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. A total of 46 constituents were identified, representing 85.6 % of the oil; limonene (46.9 %) and 1,8-cineole (7.6 %) were the main components. The free radical scavenging activity of the oil was moderate with IC50 of 11.21 mg/mL. The essential oil exhibited better inhibition of linoleic acid oxidation than ascorbic acid.

These findings indicate that the essential oil of *P.scoparius* has a potential for use as a preservative and flavorant in processed foods.

Key words: essential oil, antioxidant activity, *Pituranthos scoparius.*

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Antioxidant and Antimicrobial Properties, Phytochemical Screening and Phenolic Content of *Zizyphus jujuba Mill*. Leaves and Fruit

Nawel Gadiri [(a)], Zineb Lakache [(a)], Chafia Tigrine [(a)], and Abdelkrim Kameli [(a)]

[(a)] *Laboratoire d'Ethnobotanique et Substances Naturelles, Ecole Normale Supérieure, Kouba, Algiers, Algeria.*
nawelgadiri@gmail.comEmail

Zizyphus jujuba Mill. is a thorny rhamnaceae plant long-cultivated in the Mediterranean region. The fruit of *Z. jujuba* are commonly used as food, food additive, flavoring, and supplement for promoting health. In addition, the leaves of Z. jujuba have been also used as a folk medicine to treat children suffering from typhoid fever, furuncle and ecthyma in China.

The current study was conducted to estimate the Total Polyphenols and flavonoids content, and to evaluate antioxidant and antimicrobial capacities in ethanolic extracts of leaves and fruit of *Z. jujuba*.

Preliminary phytochemical screening using standard procedures revealed the presence of alkaloids, tannin, saponins, steroids, triterpenes and anthraquinones.

Total phenolic content (TPC) was determined by using the Folin-Ciocalteu method while total flavonoid compounds (TFC) were estimated using Aluminium chloride method.

The total phenolic contents, expressed as mg of gallic acid equivalent (GAE) per g of dry matter, was found to be 115,38 ± 0,65 mg GAE/g, 102,17±0,2 mg GAE/ g for leaves and fruit respectively. TFC were 22,2 ± 0,04 mg EQ/g for leaves and 16,68±0,04 mg EQ/g for fruit.

Antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the β-carotene bleaching test.The results showed that the analyzed extracts had free radical scavenging activities of 87,67% and 79,33% inhibition, for leaves and fruits extract respectively, the both extract showed strong capacity to inhibit lipid peroxidation with 45,62% inhibition for leaves and 63,15% inhibition for fruit.

Extracts from leaves and fruit of *Z. jujuba* were screened for their antibacterial activities against a panel of pathogenic microorganisms using agar disc diffusion method and minimum inhibitory concentration (MIC). Both leaves and fruit extracts exhibited an effective antimicrobial activity with inhibition zone diameter values ranging between 11 ± 0.33- 20.83 mm and 10,16–15 mm respectively, against most pathogenic strains tested. The MIC value of extracts ranged from 1.56 to 12.5 mg/mL.

The obtained results demonstrate that *Z. jujuba* leaves and fruit have considerable antioxidant and antibacterial activities and can be an appropriate candidate for new health-promoting resource.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Bacterial Multidrug Resistance Modulation By Natural and Semi-synthetic Flavonolignans

Katerina Rehorova[a], Simona Dobiasova[a], Lan Hoang[a], Tomas Macek[a], Vladimir Kren[b], Jitka Viktorova[a]

[a]*Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 5, CZ 166 28 Prague, Czech Republic*
[b]*Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ 142 20 Prague, Czech Republic*

rehorova@vscht.cz

Flavonolignans are important natural compounds found in various plant species (e.g. Silymarin complex in milk thistle seeds). Flavonolignans are now investigated in terms of the structural issues, intracellular signalization, and their inhibitory activities towards multi-drug resistance (MDR) proteins either in somatic cells (cancer cells) or in microorganisms[1]. We prepared by isolation, chemical and/or chemo-enzymatic derivatization new flavonolignans that were evaluated in detail for their MDR modulation activity.

The aim of the antimicrobial assay was to find compounds reducing the effective concentration of antibiotics used against different pathogenic multidrug-resistant strains of bacteria. For quantitative determination of synergistic effect of the new compounds and commercial antibiotics on the antimicrobial activity, the attention was focused on the concentration inhibiting growth of half of population (IC50) and Fractional Inhibitory Concentration (FIC) index value. The special issue in multidrug resistance topic is the resistance of biofilms, whose decrease was measured by resazurin based viability assay. Possible inhibition of quorum sensing (bacterial intercellular communication) was determined by using of several strains of *Vibrio campbellii* serving as biosensors for detection of communication molecules.

Selected flavonolignans, especially derivatives of Silybin AB and Silychristin A, turned out to be rare MDR modulators and deserve attention in further research.

The work was supported by the Czech Science Foundation project 18-00150S, mobility projects from Czech Ministry of Education, Youth and Sports INTER-COST LTC19007 (COST Action CA17104 STRATAGEM) .

References:

Chambers, C.S.; Viktorová J. *etal.* ; *J. Agric. Food Chem.* **2019** https://doi.org/10.1021 acs.jafc.9b00694

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

APPLICATION OF MOSS-TRANSPLANTATES IN BIOMONITORING

Shoxrud Fayziev[1], Elmina Gadirova[2]

DCs. Associate professor at University of University world economy and diplomacy [1] ,

PhD. Associate professor at Baku State University[2]

info@tadqiqot.uz

tadqiqot.uz

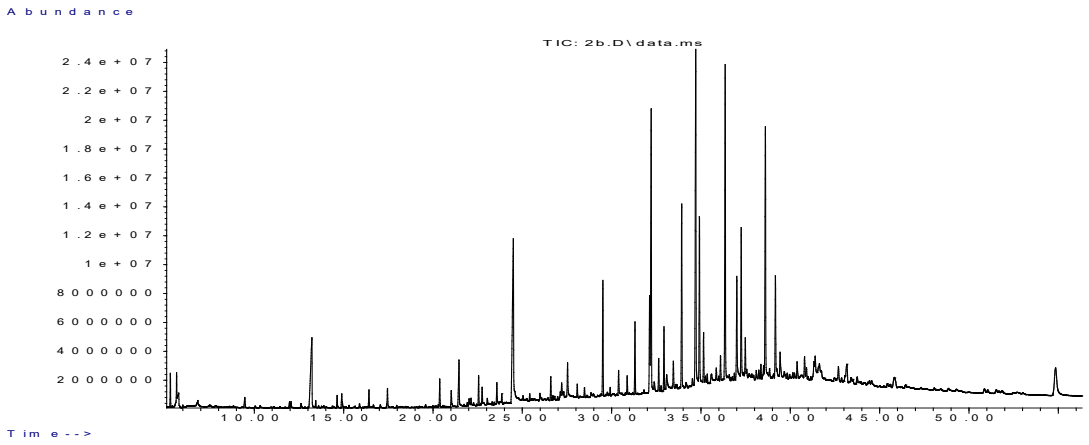
Abstract:

In order to assess air pollution in our city, for the first time, active biomonitoring was carried out using moss transplants (moss in sacs) .

In the Republic of Uzbekistan, a high population density has led to a strong anthropogenic load on natural landscapes. As is known, industrial exhaust gases, as well as motor vehicle exhaust gases, are characterized by a high content of organic substances. In order to study the content of organic substances in atmospheric air, we conducted an active biomonitoring using moss bio monitors.

Mosses – bio monitors are analogues of atmospheric filters, with the help of which any atmospheric deposition can be effectively determined [1,2]. Unlike passive, active biomonitoring involves the use of moss transplants collected in a clean, background area and exhibited in the study areas during a certain time. Samples of moss in the bags were exhibited inside and outside the city for three months. Further, the content of organic substances was studied by gas chromatography-mass spectrometry.

Fig.1. Chromatogram of moss sample exposed in the city center



In the exposed moss samples, more than 200 organic substances of various classes of compounds were identified. Among them were aldehydes, ketones, carboxylic acids, esters, saturated and unsaturated hydrocarbons. Many of these compounds have toxic, carcinogenic, mutagenic properties.

References

1. M.V. Frontasyeva, E.Steinnes and H.Harmens. Monitoring poll and air pollutants based on moss analyzes. Biomonitoring of the Air Pollution Using a Mosaic Method and the Passage of an Active Approach. New-York, USA, 2016.

2. Ryzhakova N.K., Borisenko A.L., Merkulov V.G., Rogova N.S. Control of the state of the atmosphere using moss bioindicators . Atmospheric and Oceanic Optics. 2009,1,pp. 101-104.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Modulation Of P-Glycoprotein Activity By Flavonolignans

Simona Dobiasova[a], Katerina Rehorova[a], Tomas Macek[a], Vladimir Kren[b], Jitka Viktorova[a]

[a]*Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 5, CZ 166 28 Prague, Czech Republic*
[b]*Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ 142 20 Prague, Czech Republic*

simka.dobiasova@gmail.com

Nowadays, cancer is the second most often cause of mortality after cardiovascular diseases. In many, times malignant cells exhibit less sensibility to different types of drugs. The effectivity of treatment decreases with the development of multiple drug resistance (MDR). The MDR mechanism is associated with increased expression of efflux pumps, especially P-glycoprotein (P- gp). In healthy tissues, P-gp provides transport of wide range of substances such as peptides, lipids, vitamins and metabolites. Increased expression in tumor cells causes reduced accumulation of cytostatic in the intracellular compartment. Therefore, it is important profiling of the new substances that demonstrate inhibitory activity against resistance-associated proteins. At the same time, these substances should represent a minimal level of toxicity to healthy cells. One of the possibilities of designing effective drugs is using the biological effects of flavonolignans and flavonoids. The point of submitted work was to notice the biological effects of flavonolignans and flavonoids on both tumor and non-tumor cell lines. Part of the work was to identify from among candidates appropriate samples for inhibition of P-gp transport activity. Experiments have proved the antioxidant potential and immunoprotective effects of substances. The inhibitors of P-gp ATPase activity have been identified. Flavonolignan samples had the best effect on transport activity. These agents were used to modulate the P-gp positive ovarian carcinoma cell line resistant to Adriamycin. The combination of flavonolignans and Adriamycin inhibited the transport pump, resulting in decreasing the Adriamycin concentration which is needed to inhibit the growth of resistant cells. At the same time, the natural fluorescence of some samples was monitored and after that, the cellular localization was recorded by confocal microscope. Cell nucleotide agents may be involved in altering the expression profile of ABC transporters.

The work was supported by the Czech Science Foundation project 18-00150S, mobility projects from Czech Ministry of Education, Youth and Sports INTER-COST LTC19007 (COST Action CA17104 STRATAGEM).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Antimicrobial Effect of *Eucalyptus globulus* Essential Oil in Liquid and Vapor Phases = Susceptibility of Selected Respiratory Tract Pathogens

[a] Mohamed Amine Ferhat, [b] Mohamed Nadjib Boukhatem,

[a]*Laboratoire de Recherche sur les Produits Bioactifs et la Valorisation de la Biomasse LPBVB, Ecole Normale Supérieure Vieux Kouba ENS BP 92 Kouba- Alger, Algeria.*

[b]*Laboratoire Eco-Physiologie Végétale, Département des Sciences Naturelles, Ecole Normale Supérieure de Kouba, Alger, Algeria*

Ferhatamine100@yahoo.fr

Essential oils (EO) produced by medicinal plants have been traditionally used for respiratory tract infections, and are used nowadays as ethical medicines for colds. Although several studies of *Eucalyptus globulus* essential oil (EGEO) have been reported, there are no reports describing vapour activity of EGEO against bacterial respiratory tract pathogens. The aim of this study was to test the efficacy of the Algerian EGEO against some respiratory tract pathogens by disc diffusion and vapour diffusion methods at different concentrations. Chemical composition of the EGEO was analysed by Gas Chromatography-Mass Spectrometry. Fresh leaves of *E. globulus* on steam distillation yielded 0.96 % (v/w) of essential oil whereas the analysis resulted in the identification of a total of 11 constituents, 1.8 cineole (85.8%), α-pinene (7.2%), and β-myrcene (1.5%) being the main components. By disc diffusion method, EGEO showed potent antimicrobial activity against Gram-positive more than Gram-negative bacteria. The Diameter of Inhibition zone (DIZ) varied from 69 mm to 75 mm for *Staphylococcus aureus* and *Bacillus subtilis* (Gram +) and from 13 to 42 mm for *Enterobacter* sp. and *E. coli* (Gram -), respectively. However, the results obtained by both agar diffusion and vapour diffusion methods were different. Significantly higher antibacterial activity was observed in the vapour phase at lower concentrations. *A. baumanii and Klebsiella pneumoniae* were the most susceptible strains to the oil vapour with DIZ varied from 38 to 42 mm. Therefore, smaller doses of EO in the vapour phase can be inhibitory to pathogenic bacteria. Else, the DIZ increased with increase in concentration of the oil. The present study indicates that EGEO has considerable antimicrobial activity, deserving further investigation for clinical applications.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Ecological Conditions of *Lycium barbarum* a Natural Healing Source Growing in Afyonkarahisar (Turkey) Region

Ahmet Serteser[1],

[1] *Afyon Kocatepe University, Faculty of Science and Literature, Department of Molecular Biology and Genetic, Afyonkarahisar*

e-posta: aserteser@aku.edu.tr

The study area is located within the boundaries of Afyonkarahisar in the Central-Western Anatolia of the Aegean Region and enters the B3 square according to Davis' grid system. Quaternary land covers most of the study area. The study area has a “low rainy, cold Mediterranean” bio-climate. “Eastern Mediterranean Type” precipitation regime is observed in the area. Alluvial (A), Brown (B), Hydromorphic Alluvial (H) and Colluvial (K) Large Soil Groups and reeds and marshes as land type are mostly present in the study area. *Lycium barbarum* was detected in approximately 30 localities from the study area within the Irano-Turanian floristic region.

Today, plants are utilized in different ways. Of course, as food, it is extremely important that they are widely used as clean and fresh. *Lycium barbarum* has antioxidant activity due to its high phenolic content and neutralizing cell damage caused by free radicals. Especially in recent years strengthening the immune system, delaying aging, effective against many types of Alzheimer and cancer, supported by clinical research *Lycium barbarum* fruit extract is very important to use a certain dose.

In this study, the distribution areas of *Lycium barbarum* plant in Afyonkarahisar region, which is a functional antioxidant additive in functional foods class, were determined due to different climate, soil, topography and biotic factors.

Keywords: Afyonkarahisar (Turkey), *Lycium barbarum*, Flora, Antioxidant

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Preparatıon of Pepsin Immobilized Cryogel Bioreactors

Mustafa Yeşil[a], İhsan Alacabey[b], Leyla Ercan[c], Hüseyin Alkan[d], Lokman Uzun[e], Mehmet Doğru[f]

[a]*Dicle University, Faculty of Science , Department of Chemistry,Diyarbakır*
[b]*Mardin Artuklu University, Vocational Higher School of Healthcare Studies, Mardin*
[c]*Dicle University, Institute of Science, Diyarbakır*
[d]*Dicle University, Faculty of Pharmacy, Department of Biochemistry, Diyarbakır*
[e]*Hacettepe University, Faculty of Science, Department of Chemistry, Ankara*
[f]*Dicle University, Faculty of Ziya Gökalp Education, Diyarbakır*

mdogru@gmail.com

Pepsin is an endopeptidase that breaks down proteins into smaller peptides (that is, a protease). It is produced in the stomach and is one of the main digestive enzymes in the digestive systems of humans and many other animals, where it helps digest the proteins in food.

In recent years, it has been noted that $F(ab)_2$ fragments are formed by specific cleavage of the hinge region of the whole antibody by the pepsin enzyme. The $F(ab)_2$ fragments are in the form of a monocovalent antibody harboring the complementarity-determining region (CDR) without the Fc subfragment. Antigens can be specifically identified and linked to them. In research and quality control, $F(ab)_2$ fragments are used instead of the whole structure of IgG. Furthermore, pepsin is an enzyme that can maintain its stability and activity even in the presence of high temperature and denaturing agents. Various materials such as monoliths, magnetic composite microspheres, magnetic nanoparticles, disc and cryogel bioreactors have been used to support enzyme immobilization. In recent years, cryogels have also been used as bioreactors for the production and degradation of therapeutic proteins. Bioreactors are available in the health sector for diagnostic, therapeutic purposes and for single use in biopharmaceutical production. However, the single use of bioreactors significantly increases the cost and reduces reproducibility.

In the present study, it has been studied in a continuous system and at the same time it has been suggested that an easy and effective cryogel bioreactor is made to immobilize the pepsin enzyme. For efficient and reliable design of Pepsin immobilization using cryogel bioreactor, covalent immobilization was performed between the epoxy groups of the matrix and the amino groups of Pepsin enzyme. The enzyme was characterized by immobilized cryogel bioreactor SEM and swelling test. The specific activity, substrate concentration, temperature and storage stability of the immobilized peps over a wide pH range were compared with the free pepsin in a solution using IgG susbstrate.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effects of Gamma Irradiation and Comparison of Different Extraction Methods on Sesquiterpene Lactone Yields from the Medicinal Plant Thapsia garganica L. (Apiaceae)

[a]Farid Benkaci-Ali, [b]Abir Mohamed Mohamed Ibrahim, [c]Henrik Toft Simonsen

[a]*Laboratory of Organic and Functional Analysis, Faculty of Chemistry, University of Sciences and Technologies Houari Boumediène, USTHB, BP 32 El Alia, Bâb-Ezzouar, Algiers, Algeria.*

benkaciaf@yahoo.fr

[b]*Department of Agro-Alimentary Technology and Humane nutrition, Laboratory of Chemistry, Agronomic National High School, Hassan Badi,16000, El-Harrach, Algiers, Algeria.*

[c]*Department of Biotechnology and Biomedicine, Technical University of Denmark, Søltofts Plads, 2800 Kgs. Lyngby, Denmark*

Abstract

The goal of this study was to evaluate the combined effect of microwave assisted extraction and gamma irradiation on the highly bioactive compounds found in o Algerian T. Garganica extracts. Quantification and isolation of the compounds of interest was carried out using an HPLC and Nuclear magnetic resonance NMR. The antioxidant activity extracts was determined using the two free radical scavenging assays: the 2,2-diphenyl-picryl-hydrazyl (DPPH) and the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS).

It was found that location and extraction method had significant impact on the phytochemical composition of extracts. Gamma irradiation was found to have no significant effect on the phytochemical composition of three sesquiterpene lactones, thapsigargin (Tg), nortrilobolide (Nb) and thapsigargicin (Tc) of the plant extracts as well as on their antioxidant properties.

It has been shown that microwave assisted extraction is an effective method for investigating the extraction and isolation constituents of T. garganica and the results support the fact that the gamma sterilization do not alter the chemical composition.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Cloning of The L-asparaginase Encoding Gene From A Mesophilic Bacterium

Yakup KOLCUOĞLU, Ahmet ÇOLAK
Karadeniz Technical University, Science Faculty, Department of Chemistry
yakupkolcuoglu@yahoo.com

L-asparaginase is the first therapeutic enzyme with antineoplastic properties studied extensively by researchers and scientists. Asparaginases are enzymes used as the cornerstone of the Acute Lymphoblastic Leukemia (ALL) treatment protocol. They have been used as an integral part of chemotherapy protocols in pediatric ALL and adult treatments for nearly 30 years.[1]

In this study, the gene coding for L-asparaginase from mesophilic baceterium was identified in full sequence. The L-asparaginase gene, cloned into the pET-28a (+) vector, was expressed in *Escherichia coli* BL21 (DE3) pLysS.

The study was support from by KTU-BAP (Project number FBA-2016-5615).

Reference:

Batool, T.; Makky, E.A.; Jalal, M.; Yusoff, M.M. Appl. Biochem. Biotechnol. **2016**, *178*, 900.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Cytotoxic Effects of Kaempferol in Colon Cancer Cells

Hatice Gül BATUR, İrem Mukaddes DURMUŞ, Halime Şeyma AKALIN, Serdar KARAKURT

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY

haticeglbatur@gmail.com

Cancer is a serious health problem due to mortality and morbidity. Colorectal cancer, one of the most common types of cancer, is the third leading cause of cancer-related deaths worldwide. Alternative prevention and support methods for cancer prevention, cancer treatment, or reduction of side effects are frequently tried and of interest. Herbal phenolic compounds are often tried and very popular in the treatment of cancer. One of these compounds is the plant flavonoid Kaempferol, which is a well-known anti-inflammatory, antioxidant, anti-carcinogenic compound. This study aims to clarify the cytotoxic effects of Kaempferol on the proliferation of colon cancer cells. For this purpose, the cytotoxic effects of Kaempferol on human colon cancer DLD-1 (strong metastatic) and HT-29 (invasive) cells were investigated; IC_{50} values inhibiting 50% cell growth were calculated from the sigmoidal graph of cell death and Kaempferol concentration. Kaempferol inhibited the proliferation of colon cancer cells as a dose-dependent manner and showed distinct effects in both cell. When IC_{50} values were examined, IC50 value of kaempferol was calculated as 49.55 µM for DLD-1 cell. On the other hand, there is no significant inhibition was observed against HT-29 cell. In conclusion, Kaempferol selectively inhibited proliferation of human CRC cells, which must be deeply investigated to clarify molecular mechanism of this separation. Besides, inhibition of CRC cells with Kaempferol is a valuable preliminary study for further investigations.

Keywords: Kaempferol, Colorectal Cancer, Cytotoxicity

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Eugenol plus Glycyrrhizic Acid Inhibit Azoxymethane-induced Colon Carcinogenesis via Upregulating p53 Expression

Mustafa Selim Dogru[a], Ahmet Atessahin[b], Ali Osman Ceribasi[c], Serdar Karakurt[d]

[a]*Aksaray University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology*
[b]*Firat University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology*
[c]*Firat University Faculty of Veterinary Medicine Department of Pharmacology and Toxicology*
[d]*Selcuk University Faculty of Science Department Biochemistry*

kserdar1@yahoo.com

The high mortality of cancer is one of the crucial problems in human beings especially colorectal carcinoma (CRC) with higher morbidity (2nd grade) required new prevention approaches. The aim of this study was to investigate the potential chemopreventive effects of eugenol (EU) plus glycyrrhizic acid (GA) on azoxymethane (AOM)-induced colorectal cancer in rats. In the study, 5-7 weeks age 50 Sprague Dawley male rats were used. The rats were randomly divided into five groups as follows: Control, AOM (15mg/kg bw., sc, once a week for two weeks), AOM+EU (15mg/kg bw., sc, once a week for two weeks; EU 100 mg/kg bw., gavage), AOM+GA (15mg/kg bw., sc, once a week for two weeks; GA 15 mg/kg bw., gavage), and AOM+EU+GA. All the rats except those in the control and AOM groups were treated with EU and GA for 16 weeks. At the end of the study, the colon tissues were examined in terms of aberrant crypt foci (ACF) and histopathology In addition, p53 protein expressions in colon samples were determined by Western blotting. . ACF scores were evaluated using Mann-Whitney U test while the other data were evaluated by ANOVA and Tukey test. ACF scores were expressed as median, whereas the other data were presented as mean ± standard error. The values of $p<0.05$ were accepted as statistically significant. In the colon tissues of rats treated with AOM, p53 expressions downregulated. In the histopathological examination, an increase was detected in ACF numbers and scores. The use of EU+GA decreased ACF numbers with the rates of 13.12%, compared to the AOM group ($p>0.05$). ACF were scored as small (4-6 crypts), medium (7-9 crypts) and large (≥10 crypts) ACF. There was no statistically significance ($p>0.05$) among the groups in terms of all scores, however, there was a decrease in large ACF score in all treatment groups. In addition, EU+GA upregulated p53 expression 2.12 fold ($p<0,001$) in AOM-treated rats compared to AOM group. In conclusion, EU+GA was determined to be effective against colorectal cancer.

Key words: p53; azoxymethane; eugenol; glycyrrhizic acid; ACF.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Cytotoxic Effects of Kaempferol in Colon Cancer Cells

Hatice Gül BATUR, İrem Mukaddes DURMUŞ, Halime Şeyma AKALIN, Serdar KARAKURT

Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY

haticeglbatur@gmail.com

Cancer is a serious health problem due to mortality and morbidity. Colorectal cancer, one of the most common types of cancer, is the third leading cause of cancer-related deaths worldwide. Alternative prevention and support methods for cancer prevention, cancer treatment, or reduction of side effects are frequently tried and of interest. Herbal phenolic compounds are often tried and very popular in the treatment of cancer. One of these compounds is the plant flavonoid Kaempferol, which is a well-known anti-inflammatory, antioxidant, anti-carcinogenic compound. This study aims to clarify the cytotoxic effects of Kaempferol on the proliferation of colon cancer cells. For this purpose, the cytotoxic effects of Kaempferol on human colon cancer DLD-1 (strong metastatic) and HT-29 (invasive) cells were investigated; IC_{50} values inhibiting 50% cell growth were calculated from the sigmoidal graph of cell death and Kaempferol concentration. Kaempferol inhibited the proliferation of colon cancer cells as a dose-dependent manner and showed distinct effects in both cell. When IC_{50} values were examined, IC50 value of kaempferol was calculated as 49.55 µM for DLD-1 cell. On the other hand, there is no significant inhibition was observed against HT-29 cell. In conclusion, Kaempferol selectively inhibited proliferation of human CRC cells, which must be deeply investigated to clarify molecular mechanism of this separation. Besides, inhibition of CRC cells with Kaempferol is a valuable preliminary study for further investigations.

Keywords: Kaempferol, Colorectal Cancer, Cytotoxicity

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Nanofibers and Their Applications

Kutbettin Arslan, Gulten Okmen

Mugla Sitki Kocman University, Faculty of Science, Department of Biology, Mugla, TURKEY

gultenokmen@gmail.com

Nanofibers have become an increasingly important research topic in recent years due to their micro and nano structural properties that allow the development of materials with special applications. Furthermore, large surface area and small pore size that they have, increases the importance of these fibers. Nanofibers, with diameters in the nanometer range, possess larger surface areas per unit mass. These fibers, permit easier addition of surface functionalities compared with polymer microfibers. Hence, polymer nanofiber are being considered for use as filters, scaffolds for tissue engineering, protective clothing, reinforcement in composite materials and sensors. There are many methods of nanofiber production, they are as follows; electro-spining, phase separation, drawing, self-assembly, and template synthesis etc. Although there are many methods of fabricating nanofibers, electro-spinning is perhaps the most versatile process. However, what makes electro-spinning different from other nanofiber fabrication processes is its ability to form various fiber assemblies. In recent years, electro-spinning nanofibrous membranes have been used to the wound dressing, tissue engineering, and transportation of drugs areas. Because nanofibers have different biological activity, biological compatibility, biodegradability, low toxicity, and naturally large surface area. This review provides an basic information to nanofibers, production methods, bio-based polymers, and their applications.

Key words: Nanofiber, electrospinning, bio-based polymer, application

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Comparative analysis of close outcomes of total mesorectal excision (TME) in malignant derivatives

Azimov E.H.

Azerbaijan Medical University, I Department of Surgical Diseases

elman-azimov@mail.ru

*Abstract***.** Laparoscopic total mesorectal excision (TME) in malignant masses of rectum with different localization is going spread widely over the world. But, the final point was not put to the issue of open or laparoscopic operations. At present, close results of laparoscopic and open TME are analyzed in research work.

Material and methods of research. Clinical materials of state medical institution (Clinical Medical Centre) and private medical centre (Elmed medical centre) were used in research work. 47 of 103 patients with the diagnosis of cancer of rectum were included in laparoscopic group, 56 of them were included in open group. Patients underwent to general clinical examinations, MRT of pelvis, CT examination of chest and abdomen, colonoscopy (biopsy), CEA, determination of C19-9 tumor markers, etc.

*Result and discussion of research***.** The continuation period of operations was expressed with high figures in patients included in laparoscopic group during our comparative analysis (330±60 minutes and 275±35 minutes). Laparoscopic technology preferred to open operations in other images. The quantity of blood lost during operation was 245±135ml in laparoscopic group, 340±145ml in open group, need for narcotic analgesics was 66.5±1.8mg in laparoscopic group, 115.4±2.4mg in open group, commencement period of intestinal peristalsis was 32.8±1.5 hours in laparoscopic group, 61.2±1.8 hours in open group, enteral nutrition was 47.5±2.4 hours in laparoscopic group, 69.7±3.8 hours in open group, the first defecation was 4.7±1.1 days in laparoscopic group, 5.9±1.3 days in open group, total days for staying in stationary and postoperative ward days were 18.5±4.7 days in laparoscopic group, 22.4±5.6 days and 13.14±4.2 days in open group[1,2].

References

1.Kusters M., Wallner C., Lange M.M. et all. Origin of presacral local recurrence after rectol cancer treatment . Br/ J,Surgery, 2010.

2. Lliang J.T., Lai H.S., Lec P.H. et all. Laparascopic pelvic autonomic nerve preserving surgery for sigmota colon cancer . Ann Surg Oncol., 2018, 6, 1609-1616.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Association Between CTGF rs9402373 Polymorphism and Pseudoexfoliation Syndrome

Canan Koçan-Akçin [a], Birsen Can Demirdöğen [a], Gökhan Özge [b], Tarkan Mumcuoğlu [b]

[a] *TOBB University of Economics and Technology, Department of Biomedical Engineering, Ankara, Turkey*

[b] *University of Health Sciences, Faculty of Medicine, Gülhane Education and Research Hospital, Ophthalmology Unit, Ankara, Turkey*

birsencan.demirdogen@gmail.com

Pseudoexfoliation syndrome (PES) is an extracellular matrix disorder characterized by the production and accumulation of abnormal fibrillar materials in many ocular tissues[1]. Pseudoexfoliation material can increase the intraocular pressure by blocking the channels that drain the aqueous fluid, causing glaucoma and vision loss[2,3]. Since PES is characterized by over-synthesis of microfibrillar components, growth factors may play a role in the pathophysiology of PES. Connective tissue growth factor (CTGF) is a protein expressed in several tissues, including the anterior chamber of the eye[4]. One of the genetic polymorphisms in the CTGF gene, rs9402373 C/G single nucleotide polymorphism (SNP), is an intron variant and may affect the stability of the transcript[5]. The aim of this study was to investigate if there is any association between rs9402373C/G polymorphism and PES.

Study population consisted of 214 PES patients and 214 controls. Blood samples were collected by Gülhane Training and Research Hospital, Department of Ophthalmology, Ankara. Genotypes were assigned by real-time PCR (RT-PCR) using Taq-man genotyping kits. Genomic DNAs were isolated from whole blood samples using Qiagen DNA isolation kit. In the study population, 140 PES patients and 142 controls had the homozygous wild type genotype (CC), while 69 PES patients and 66 controls had heterozygous genotype (CG) and 5 PES patients and 6 controls had homozygous polymorphic genotypes (GG). rs9399005 C/G polymorphic allele G frequency was found to be 0.185 in PES and 0.182 in controls (P = 1,000). This work did not point out a role for this SNP in the risk for PES. rs9402373C/G was studied first time for the risk for PES. Moreover, allele frequencies of this SNP were determined for the first time in Turkish population.

Acknowledgment: This study was supported by TUBITAK (115S360).

References:

Streeten, B. W.; Li, Z. Y.; Wallace, R. N.; et. al. *Arch Ophthalmol.* **1992**, *110,* 1757–1762.
Yalvaç, I.; Önal, M. Temel Göz Hastalıkları, Glokom, 1. Baskı. Editörler: Aydın, P., Akova, Y. A. A kara: Güneş Kitabevi, 2001.
Ritch, R.; Schlotzer-Schrehardt, U. *Survey of Ophthalmology.* **2001**, *45*(4), 265-315.
Browne, J. G.; Ho, S. L.; Kane, R.; Oliver, N.; Clark, A. F.; O'Brien, C. J.; Crean, J. K. *Investigative Ophthalmology and Visual Science.* **2011,** *52*(6), 3660-6.
Dessein, A.; Chevillard, C.; Arnaud, V.; et al. *Journal of Experimental Medicine*. **2009,** *206*, 2321 2328.

2EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In Vitro Antioxidant, Anti-inflammatory and Cytotoxic Potential of Thyme (*Thymus vulgaris*) Essential Oil and its Related Terpenes on Tumor Cell Lines

Mohamed Nadjib BOUKHATEM [123*], Noureldien H.E. DARWISH [1], Thangirala SUDHA [1], Hanady NADA [1], Siham BAHLOULI [2], Henni CHADER [4], Mohamed Amine FERHAT [3], Abdelkrim KAMELI [3], Yasmine BENALI [5], Shaker A. MOUSA [1]

1/ The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, Rensselaer, NY, USA.

2/ Département de Biologie et Physiologie Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université – Saad Dahlab – Blida 1, Blida, Algeria.

3/ Laboratoire Ethnobotanique et Substances Naturelles, Ecole Normale Supérieure, Kouba, Alger, Algeria.

4/ Service Pharmaco-Toxicologie, Laboratoire National de Contrôle des Produits Pharmaceutique, Dely-Brahim, Algeria.

5/ Laboratoire d'Anatomie et Cytologie Pathologiques Vétérinaires, Institut Pasteur d'Algérie, Algeria.

* Corresponding Author: mn.boukhatem@yahoo.fr

ABSTRACT

Introduction: In recent years, essential oils have been reported to possess interesting anti-tumor, anti-mutagenic and anti-carcinogenic activities against various cancer cells. Therefore, we investigated the *in vitro* anti-inflammatory, antioxidant and cytotoxic potential of *Thymus vulgaris* essential oil (TVEO) and some related terpenes (Thymol, Carvacrol and Linalool) on cancer cell lines.

Methods: The cytotoxicity assay was done with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to investigate changes in mitochondrial/non-mitochondrial dehydrogenase activity and to determine the potential anti-proliferative property of the TVEO and related terpenes against two human cancer cell lines: Human Breast Adenocarcinoma (MDA-MB 231) and Melanoma M3. Anti-inflammatory activity was assessed with *in vitro* (Human Red Blood Cell Membrane Stabilisation) and *in vivo* (xylene induced-ear edema) tests.

Results: The chemical composition of TVEO was determined with Gas Chromatography and revealed the presence of six compounds. Carvacrol was found to be the major component (77.4%). Linalool and Carvacrol were detected in minor quantities (0.88% and 0.52%, respectively).

Antioxidant activity was evaluated with a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and metal chelating ions test. The percentage inhibitions were concentration dependent with IC50 value of TVEO of 1.4 µg/mL scavenging activity in DPPH free radical, while those of BHA and ascorbic acid were 0.44 µg/mL and 2.12 µg/mL, respectively. Topical anti-inflammatory potential of TVEO was also explored *in vivo* and exhibited a potent anti-inflammatory effect at all doses (100, 10 and 2 mg/kg), which were statistically similar to the positive control (Diclofenac Sodium). This activity was also confirmed at the cellular level with a histopathology analysis.

The MTT assay showed that the TVEO exhibited significant dose-dependent growth inhibition in the 50–200 mM dilution range. Carvacrol and Thymol showed better results compared to Linalool, particularly for breast cancer cells.

Conclusion: Higher concentration of Carvacrol and/or synergistic effect of the overall composition were probably responsible for the efficacy of TVEO against the tested cancer cells. TVEO may be a potential source of natural anti-cancer compounds and play an important role in human health.

Keywords: Anticancer Activity; Breast Cancer; Melanoma; Thyme Essential Oil; *in vivo* Anti-inflammatory Effect; Antioxidants.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In vitro Cytotoxicity and *Ex ovo* Anti-Angiogenic Activity of Lemongrass (*Cymbopogan citratus*) against Breast Carcinoma Cell Line

Mohamed Nadjib BOUKHATEM [123*], Noureldien H.E. DARWISH [1], Thangirala SUDHA [1], Hanady NADA [1], Mehdi RAJABI [1], Shaker A. MOUSA [1]

1/ The Pharmaceutical Research Institute, Albany College of Pharmacy and Health Sciences, 1 Discovery Drive, Rensselaer, New York, USA.

2/ Département de Biologie et Physiologie Cellulaire, Faculté des Sciences de la Nature et de la Vie, Université – Saad Dahlab – Blida 1, Blida, Algeria.

3/ Laboratoire Ethnobotanique et Substances Naturelles, Ecole Normale Supérieure, Kouba, Alger, Algeria.

Corresponding author: Dr. Boukhatem M.N.; mail: mn.boukhatem@yahoo.fr

ABSTRACT

Background: The search for new drugs that display activity against several types of cancer has become one of the most interesting subjects in the field of natural products research.

Methods: *Cymbopogon citratus* essential oil (CCEO) was isolated using a steam distillation method and tested for cytotoxicity activity and anti-angiogenic activity using a breast carcinoma cell line (MDA-MB 231). Cytotoxicity activity was determined by using the MTT assay and the anti-angiogenic activity was determined by using a chorioallantoic membrane of fertilized hen's egg (HET-CAM assay) and compared to a standard angiogenic substance.

Results: The chemical composition of CCEO was determined with gas chromatography and revealed that citral was the major compound (60.3%), followed by menthyl acetate (9.5%) and menthone (5.5%).

The irritation potential test in the Red Blood Cell (RBC) system cellular model allowed the quantification of adverse effects of CCEO on the plasma membrane of RBC and the consequent release of hemoglobin (hemolysis), which enables the determination of the irritation degree of the CCEO. CCEO manifested high hemolytic activity (H50 = 0.4614%) and a great attention will take with greatest concentrations.

The *ex ovo* test using the HET-CAM assay was used for the determination of the anti-angiogenic effect of CCEO and revealed that oil-treated CAMs branched into more multi-stage capillaries and more abundant neo-vasculatures. Our results in the HET-CAM assay suggested a moderate anti-angiogenic effect (angiogenesis index branch points = 60±10) of the CCEO at the tested concentrations (2 µL/pellet) in comparison with basic Fibroblast Growth Factor (*140*±9) at the dose of 1 µg/mL.

Breast cancer cells were treated with CCEO concentrations ranging from 4 to 0.03% for 24 and 48 h. CCEO has a potential cytotoxic effect on MDA-MB 231 with IC50 value of 0.063%.

Conclusion: Data obtained in these experiments suggest that further investigations are warrented using CCEO on various cancer cell lines due to its anti-angiogenic effects observed in this work.

Keywords: Angiogenesis; Anticancer effect; Breast Cancer; *Cymbopogon citratus* Essential Oil; Phytomedicine.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Purification and Characterization of Carbonic Anhydrase from Anchovy Gills and Investigation of Inhibition Effects of Some Heavy Metal Ions on the Enzyme Activity

Nuran Arslan[a], Elif Duygu Kaya[b], Hakan Söyüt[c], Şükrü Beydemir[d]

[a] *Ataturk University, Faculty of Science, Department of Chemistry, Erzurum, Turkey*

[b] *Iğdır University, Faculty of Engineering, Department of Food Engineering, Iğdır, Turkey*

[c] *Uludağ University, Education Faculty, Department of Primary Education, Bursa, Turkey*

[d] *Anadolu University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Eskişehir, Turkey*

hakansoyut@uludag.edu.tr

The carbonic anhydrase enzyme (CA) reversibly catalyzes the hydration of carbon dioxide and the dehydration of bicarbonate in living organisms. This enzyme has proven important roles in fish gills. It is thought to be involved in gas-exchange, maintenance of acid-base balance, osmoregulation, ion regulation and removal of waste products in nitrogen metabolism.[1] In the present study, carbonic anhydrase (CA)) enzyme was purified from anchovy (*Engraulis encrasicolus*) gills tissue with a specific activity of 1473 EUxmg^{-1} and a yield of 26.32 % using Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography, respectively. The overall purifications from anchovy (*Engraulis encrasicolus*) gills tissue CA enzyme was approximately 39-fold. SDS-gel electrophoresis was applied to determine the purification of the enzyme and subunit molecular weights. It was observed as a single band. MW was approximately 25.1 kDa. The kinetic and characteristic properties of CA such as K_m and V_{max} were determined. The inhibitory effects of different heavy metals (copper, silver, and lead) on anchovy CA activity were investigated by using esterase method under in vitro conditions. The heavy metal concentrations, inhibiting 50% of gills tissue CA enzyme activity (IC_{50}), were obtained for CA enzyme from gills tissue. K_i values and inhibition types for these inhibitors (copper, silver, and lead) were also calculated by using esterase method, from Linewaewer-Burk graphs. K_i values were 0.264 mM for lead (Pb^{2+}), 3.98 mM for copper (Cu^{2+}), and 0.0064 mM for silver (Ag^{+}). Silver was competitive, and the others were noncompetitive.

References (optional):

Kaya, E. D.; Söyüt, H.; Beydemir, Ş. *Environ. Toxicol. Pharmacol.* **2015**, 39, 825-832.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Photocatalytic Hydrogen Production from Plant Based Carbon Quantum Dot Structures

Munevver Tuna Genc[a], Gizem Yanalak[b], Gulsin Arslan[b] , Imren Hatay Patir[a]

[a]Selcuk University, Faculty of Science, Department of Biotechnology, Konya, Turkey

[b]Selcuk University, Faculty of Science, Department of Biochemistry, Konya, Turkey

munevver1tuna@gmail.com

Carbon quantum dots (CQDs) have attracted growing interest due to their unique physicochemical, optical and electronic properties, and their superior luminescent properties which make them excellent photosensitizers for TiO2. Those tremendous properties have displayed in numerous fields such as biosensing, bioimaging, drug delivery, optoelectronics, photovoltaics and photocatalysis. CQDs have recently been introduced introduced into catalytic applications.[1] In addition, owing to the unique PL behavior, CQDs are potentially considered to be an efficient componet in the construction of high-performance photocatalysts.[2] This study presents the green-synthesis of CQDs which obtained from *gingko biloba* by using microwave technique due to easy, time-saving, cost-effective, non-toxic, and environmental friendly. CQDs/TiO_2 composite was prepared via a facile one step hydrothermal method. Characterization of the samples was carried out by Transmission electron microscopy (TEM), High-resolution TEM and Fourier transformed infrared (FT-IR) spectroscopy. CQDs/TiO_2/Pt composite exhibited improved photocatalytic hydrogen evolution under visible light ($\lambda > 450$ nm) irradiation in the aqueous solution by using TEOA as a sacrificial electron donor compared to CQDs/TiO_2 composite.

References :

Wang, R., et al. (2017). "Recent progress in carbon quantum dots: synthesis, properties and applications in photocatalysis." Journal of Materials Chemistry A **5**(8): 3717-3734.

Yu, H., et al. (2014). "Carbon quantum dots/TiO 2 composites for efficient photocatalytic hydrogen evolution." Journal of Materials Chemistry A **2**(10): 3344-3351.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Preparation of a New Biosensor for Bisphenol A Determination

Hasan Koçak, Onur Can Bodur, Elvan Hasanoğlu Özkan, Nurşen Sarı, Fatma Arslan

Gazi University, Institute of Sciences, Department of Chemistry, Ankara, TURKEY

onurcanbodur@gazi.edu.tr

Bisphenol A (BPA), is an industrial chemical substance as a monomer in the production of epoxy resins and polycarbonates and is used as raw material in food and drink packaging[1,2]. BPA contamination in food usually occurs as a result of migration from the packages that contains BPA. BPA affects the endocrine system primarily. This endocrine defecting chemical substance was many affects on the activity of endogenous eustrogens and the androgen system. BPA, also may play a role in thyroid hormone disfunctions, central nervous system function disorder and immune supression[1].

In this study, a novel biosensor was prepared for bisphenol A determination. For this purpose firstly, a carbon paste electrode modified with PAMAM-Sal-Pt(II)-active carbon composite and tyrosinase enzyme (polyphenol oxidase) were prepared. For PAMAM-Sal-Pt(II); Synthesized and characterized from PAMAM, Salyclaldehyde and $K_2[PtCl_4]$ (Figure). And then, Tirosinase enzyme was immobilized onto the modified carbon paste electrode surface by cross-linking with glutaraldehyde. Bisphenol A compounds form quinone compounds in oxygenated environment were catalyzed by tyrosinase enzyme. Bisphenol A determination was made on the basis of the reduction of the reaction product quinone on the modified carbon paste electrode surface at **-0.15 V.** Then, appropriate working conditions of the prepared bisphenol A biosensor were investigated. For this purpose, the linear working range of the enzyme electrode was determined. The effect of pH and temperature on the amperometric response of the biosensor were investigated. In addition, reusability and shelf life were determined. The interference effects of some cations, anions and molecules in bisphenol A determination were investigated.

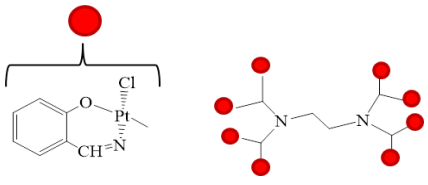


Figure Structure of PAMAM-Sal-Pt(II)

References:

1)Buket ER, Belgin SARIMEHMETOĞLU, The assesment of bisphenol A presence in Foods, Vet Hekim Der Derg, 82(1): 69-74, 2011.

2)Tsai WT, Human health risk on environmental exposure to bisphenol-A: A Review. J Environ Sci Heal C. 24, 225-55,2006.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Inhibition Effects of Zinc (Zn^{2+}), Lead (Pb^{2+}), and Silver (Ag^{+}) Ions on Carbonic Anhydrase from Whiting Gills

Nuran Arslan[a], Elif Duygu Kaya[b], Hakan Söyüt[c], Şükrü Beydemir[d]

[a] *Ataturk University, Faculty of Science, Department of Chemistry, Erzurum, Turkey*

[b] *Iğdır University, Faculty of Engineering, Department of Food Engineering, Iğdır, Turkey*

[c] *Uludağ University, Education Faculty, Department of Primary Education, Bursa, Turkey*

[d] *Anadolu University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Eskişehir, Turkey*

hakansoyut@uludag.edu.tr

Carbonic anhydrase (EC 4.2.1.1, CA) catalyzes the reversible reactions of CO_2 and H_2O in living organisms. It plays a role in these biochemical processes such as acid-base balance, respiration, carbondioxide and ion transport, bone resorption, ureagenesis, gluconeogenesis, lipogenesis and electrolyte secretion.[1] In the present study, carbonic anhydrase (CA)) enzyme was purified from whiting (*Merlangius merlangus euxinus*) gills tissue with a specific activity of 2800 EUxmg^{-1} and a yield of 23.33 % using Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography, respectively. The overall purifications from whiting *(Merlangius merlangus euxinus)* gills tissue CA enzyme was approximately 192-fold. SDS-gel electrophoresis was applied to determine the purification of the enzyme and subunit molecular weights. It was observed as a single band. MW was approximately 29.5 kDa. The kinetic and characteristic properties of CA such as K_m and V_{max} were determined. The inhibitory effects of different heavy metals (zinc, silver, and lead) on anchovy CA activity were investigated by using esterase method under in vitro conditions. The heavy metal concentrations, inhibiting 50% of gills tissue CA enzyme activity (IC_{50}), were obtained for CA enzyme from gills tissue. K_i values and inhibition types for these inhibitors (zinc, silver, and lead) were also calculated by using esterase method, from Linewaewer-Burk graphs. K_i values were 0.138 mM for lead (Pb^{2+}), 0.716 mM for zinc (Zn^{2+}), and 0.0477 mM for silver (Ag^{+}). Zinc (Zn^{2+}), lead (Pb^{2+}) and silver (Ag^{+}) were noncompetitive.

References (optional):

Kaya, E. D.; Söyüt, H.; Beydemir, Ş. *Environ. Toxicol. Pharmacol.* **2013**, 36, 514–521.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

"Interaction of methyltransferase Set7/9 and γ-catenin in human tumor cell models"

Zharova Arsenia, Shuvalov Oleg, Nikolay Barlev

Institute of cytology RAS, St.Petersburg, Russia

von_kleist@mail.ru

The study of lysine methyltransferase is particularly relevant in the context of regulation of non-histone proteins involved in the control of proliferation and cell cycle. Defects of this class of enzymes are characteristic of many malignant tumors. The Set7/9 methyltransferase is an important regulator of oncogenesis. One of non-histone proteins interacting with Set7/9 discovered in our laboratory is γ-catenin (plakoglobin) — a homologue of β-catenin, a key element in signal transduction mediated by Wnt. This research is aimed at studying the mechanisms of interaction between Set 7/9 methyltransferase and γ-catenin in human tumor cell models. We confirmed the physical interaction between γ-catenin and methyltransferase Set7/9. It was found that the *SETD7* gene knockout causes changes in the expression of *CCND1*, *CTNNB1* and *JUP* genes, as well as the amount of their corresponding protein products. It was shown that the *SETD7* gene knockout caused changes in the intracellular localization of β - and γ-catenins. When the *SETD7* gene is knocked out, the migration potential of the HEK 293T cell line does not change, but the migration potential of A549 cells increases, which apparently indicates a different effect of Set7/9 on cells depending on the context.

This work was supported by Grant from the Russian Goverment 14.W03.31.0029.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Photocatalytic degradition of dyes from environmental water by composite membranes

Canan Armutcu[a], Çağrı Tartan[a], Erdoğan Özgür[a,b], Demet Baykan Özer[a], Lokman Uzun[a]

[a]*Hacettepe University, Faculty of Science, Department of Chemistry, Ankara, Türkiye*
[b]*Hacettepe University, Advanced Technologies Application and Research Center, Ankara, Türkiye*

tntartan@gmail.com

Dyes discharged to the environment after industrial production processes cause a great danger for health and food chain. Different techniques are applied for the removal of dyes and color removal resulting from adsorption and photocatalytic degradation has recently attracted great attention in terms of ease of application and cost. Vanadium is an element with different oxidation states and capable of forming complexes having photocatalytic features. In particular, stable complexes with amino acids such as histidine and cysteine have been included in the literature.

Herein, vanadate ion complex incorporated composite membranes were synthesized. Vanadate ions were complexed with N-methacryloyl-L-cysteine (MAC), polymerizable derivative of L-cysteine amino acid, in order to incorporate the complex directly into the polymeric backbone. Composite membranes were characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectrophotometer (FTIR). Photocatalytic degradation of dyes were studied in the presence and absence of UV (in the dark and daylight as two different conditions). While the presence of UV significantly increased photocatalytic activity, any change in the spectrum of the dyestuff wasn't observed after the interaction in the dark. In addition, photocatalytic activity increased by increasing amount of vanadate complex. Determination of the removal of dyes by adsorption or by photocatalytic degradation was examined via gas chromatography/ mass spectrometry (GC/MS) and it was found that photocatalytic degradation led to dye removal. The composite membrane protects their activity by 92.58 % after five consecutive photocatalysis-washing-equilibrium cycles.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Development of 2-Methacryloyloxyethyl Phosphorylcholine (MPC) Based Cryogel Membranes

Erdoğan Özgür

Advanced Technologies Application and Research Center, Hacettepe University, Ankara, Turkey

erdoganozg@gmail.com

Synthesis of artificial/natural polymeric biomaterials having resistance to nonspecific protein adsorption, blood coagulation and bacterial adhesion has attracted great attention because nonspecific adsorption of proteins and biomolecules leads to unfavorable biological reeponses such as blood clotting, inflammation, biofilm formation, cell adhesion, and cell differentiation. A zwitterionic phosphorylcholine (PC) group (endows the cell membrane with ideal biocompatibility) in the side chain of MPC is responsible for biologically inert functions, especially in resistance to protein adsorption. Due to the reactivity of methacrylate, MCP can be easily be copolymerized to develop numerous materials with with tunable properties via various polymerization techniques, so have a wide range of applications in biomedical fields[1-3]. Thus, we aimed to develop bio-inspired, efficient and environmentally friendly cryogel membranes containing MPC. In this study, it is aimed to develop MCP containing cryogel membranes that can be applied in blood-contacting materials. Cryogel membranes were synthesized in semi-frozen medium by free radical polymerization initiated by N,N,N′,N′-tetramethylene diamine (TEMED) and ammonium persulfate (APS) pair in an ice bath. The characterization of the MCP based cryogel membranes was carried out by SEM, FTIR and BET.

References (optional):

Zhang, J.; Yang, B.; Jia, Q.; Xiao, M.; Hou, Z. *Polymers*, **2019**, 11, 860.
Goda, T.; Ishara, K.; Miyahara, Y. *J. Appl. Polym. Sci*. **2015**, 41766, 1.
Ishara, K. *Sci. Technol. Adv. Mat.* **2000**, 1, 131.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Modulation of CYP1A and Antioxidant Enzyme Activities in rainbow trout (*Oncorhynchus mykiss*) by Benzophenone-3 and N,N-Diethyl-m-toluamide

Azra Bozcaarmutlu[a], Ahmet Günbür[a], Volkan Yıldırım[a], Canan Sapmaz[a], Hakan Türker[b]

[a]*Bolu Abant Izzet Baysal University, Faculty of Arts and Science, Department of Chemistry, Bolu, Turkey*

[b]*Bolu Abant Izzet Baysal University, Faculty of Arts and Science, Department of Biology, Bolu, Turkey*

bozcaarmutlu_a@ibu.edu.tr

Nowadays sunscreens and insect repellents are the two important cosmetic products that are increasingly used especially during the summer seasons all around the world. Benzophenone-3 (BP-3) is a UV filter found in sunscreen agent. N,N-Diethyl-m-toluamide (DEET) is the main ingredient of insect repellents. Despite the frequent occurrence of both of these compounds in aquatic environments, the effect of them on xenobiotic metabolizing enzyme activities has not been determined in aquatic organisms. The aim of this study is to determine the effect of benzophenone-3 and N,N-diethyl-m-toluamide on CYP1A-associated 7-ethoxyresorufin-O-deethylase activity (EROD) and antioxidant enzyme activities, catalase (CAT) and glutathione reductase (GR) in rainbow trout (*Oncorhynchus mykiss*). For this purpose, rainbow trout samples were treated with two different doses of BP-3 and DEET (100 µg/L and 500 µg/L). Fish samples were randomly divided into eight groups namely control, solvent control (DMSO), 100 µg/L BP-3, 100 µg/L DEET, 100 µg/L BP-3 + 100 µg/L DEET, 500 µg/L BP-3, 500 µg/L DEET and 500 µg/L BP-3 + 500 µg/L DEET. Fish were kept in 200 L aquarium and treated with indicated amount of chemicals individually or in combinations for 9 days. Fish were killed by decapitation. EROD activities were measured in liver microsomes and CAT and GR activities were measured in liver cytosols. EROD activities of 100 µg/L BP-3, 500 µg/L BP-3 and 500 µg/L BP3 + 500 µg/L DEET treated groups were significantly different from control and solvent control. EROD activities of 100 µg/L BP-3 + 100 µg/L DEET group were significantly different from all groups. CAT activities of 100 µg/L DEET group were significantly different from all groups. GR activities of 500 µg/L BP-3 group were significantly different from all groups. The results of this study indicate that BP-3 and DEET individually and/or in combinations modified CYP1A and antioxidant enzyme activities in rainbow trout.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Computational Construction TbpA - TbpB - hTF Triple Complex

[a]Gizem Nur DURAN, [b] Mehmet ÖZBİL

[a]*Marmara University,Department of Chemistry, Kadıköy, İstanbul, TURKEY*

[b]*İstanbul Arel University, Department of Molecular Biology and Genetics, Büyükçekmece, İstanbul, TURKEY*

gizemduran@marun.edu.tr

Neisseria meningitidis, commonly referred to as meningococcus, is a Gram-negative bacterium that causes meningitis and meningococcal diseases that threaten human life 1 . As a human pathogen, *Neisseria meningitidis* is a major problem in children and adults. *Neisseria meningitidis* requires iron Fe3+ for its survival. Therefore, it binds to human cell and transfers Fe3+ ions in human serum transferrin (hTF) protein to its structure 2 . This transfer actualized by two large surface in cell membrane, TF binding proteins A (TbpA) and B (TbpB). TbpB is located outside the cell connectedly the cell membrane. TbpA is the membrane protein. The TbpA-TbpB-hTF triple complex is formed before the transfer to occur.

In 2012, Noinaj et al. *Neisseria meningitidis* bacteria that caused by meningitidis, discovered TbpA and TbpB proteins separately and the X-ray crystal structures of the TbpA-hTF binary complex 3. This study proposed that formation of TbpA-TbpB-hTF triple complex, Fe3+ ion was acquired by the bacterium through TbpA beta sheeted ion channel. However, the lack of three-dimensional structure of the triple complex leaves this suggestion incomplete.

In this study, the three-dimensional structure of the TbpA-TbpB-hTF ternary complex is elucidated. For this purpose, the extracellular protein TbpB and hTF, also located outside the cell and containing two Fe3+ ions, were placed separately in water-filled simulation boxes and subjected to 100 ns long classical molecular dynamics (MD) simulations. Similarly, membrane protein TbpA was placed in DPPC membrane model and subjected to 100 ns classical md simulations. In the next step, the average structures obtained from these simulations were combined with molecular docking simulations for the first time in the literature to reveal the three-dimensional structure. Thus, the interactions that lead to the formation of complexes have been revealed in atomic dimension and the basis for the studies of drug molecule design that will prevent these interactions have been formed.

References :

1. Hagman M, Forslin L, Moi H, Danielsson D. *Neisseria meningitidis* in specimens from urogenital sites. Is increased awareness necessary? Sex Transm Dis 1991;18:228– 32. CrossRefexternal icon PubMedexternal icon.

2. Gray-Owen, S.D. and A.B. Schyvers, *Bacterial transferrin and lactoferrin receptors.* Trends in Microbiology, 1996. **4**(5): p. 185-191.

3. Anderson, M. S., M. P. Glode, and A. L. Smith. 1998. Meningococcal diseases, p. 1143–1156. In R. D. Feigin and J. D. Cherry (ed.), Textbook of.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Synthesis of Thermoresponsive Hydrogels with Magnetic Nanoparticles for Controlled Release of Naringin

Gülşah Sevimli [a], Özgür Gül [a], Pınar Çakır Hatır [b]

[a] *Department of Genetics and Bioengineering, Istanbul Bilgi University, Turkey*

[b] *Department of Biomedical Engineering, Istanbul Arel University, Turkey*

gulsah_sevimli@hotmail.com

Development of novel controlled drug release systems is one of the topics that researchers have been focusing on for a long time. The main principle of controlled drug release systems is to release drug molecule at the target area at the desired time. Hydrogels, which can be described as hydrophilic cross-linked polymer networks, are capable of responding to an external stimulus by swelling or shrinking. They are often used to design drug release systems.1 Magnetic nanoparticles are often preferred in many biomedical applications as they are biocompatible and can be controlled by an external magnetic field.2 Naringin is a flavanone glycoside found in abundance within the grapefruit. 3 Studies have shown that naringin has many pharmaceutical effects such as anti-inflammatory, antioxidant, anti-cancerogenic effects, as well as it can lower lipid level and lower cholesterol in the blood.

In this study, it is aimed to synthesize thermoresponsive hydrogels containing naringin with magnetic nanoparticles to be used in hyperthermia for cancer treatment and to investigate the release profiles of hydrogels depending on the external temperature. At first, thermoresponsive hydrogels with different types of crosslinkers and monomer concentrations were synthesized by photopolymerization using N-Isopropylacrylamide (NIPAM) as a monomer, ethylene glycol dimethylacrylate (EGDMA) and N,N-ethylene bisacrylamide (EBAM) as crosslinkers. The structural characterizations of hydrogels were performed by FTIR analyses. Drug release profiles of hydrogels were investigated by UV/vis s pectrophotometer at 284 nm at room temperature (25 ° C) and at body temperature (37 ° C). As a result, it was observed that the hydrogels synthesized with EGDMA were more effective for naringin release. After this step, magnetic nanoparticles having different functional groups (-OH and -NH 2) were loaded into the hydrogels and spectrophotometric measurements at 25 ° C and 37 ° C were carried out to evaluate release profiles. As a conclusion, it was found that magnetic nanoparticles with amine functionality (-NH 2) were more effective for drug

release.

References:

1) Koca, E. I., Evrensel, C., Cayli, G., Hatir, P. C. *E lectric Electronics, Computer Science, Biomedical Engineerings' Meeting (EBBT)* , IEEE, **2017** .

2) Jalili, N. A., Muscarello, M., Gaharwar, A. K., *Bioeng. Transl. Med.,* **2016** , *1* (3), 297.

3) Gorinstein, S., Leontowicz, H., Leontowicz, M., Krzeminski, R., Gralak, M., Delgado- Licon, E., Trakhtenberg, S. *J. Agric. Food Chem.* , **2005** , 53(8), 3223.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Phenolic compounds of *Chaerophyllum bulbosum*

Gülsen Tel-Çayan[a], Ebru Deveci[b], Fatih Çayan[a], Mehmet Emin Duru[b]

[a]*Muğla Sıtkı Kocman University, Muğla Vocational School, Department of Chemistry and Chemical Processing Technologies, Muğla, TURKEY*

[b]*Muğla Sıtkı Kocman University, Faculty of Science, Department of Chemistry, Muğla, TURKEY*

**Corresponding Author: gulsentel@mu.edu.tr*

Studies on aromatic plants for the preparation of alternative pharmaceutical components in the management of various diseases are increasing. Among the bioactive compounds present in aromatic plants, phenolics are one of the most important and probably the main candidates responsible for the most health beneficial properties (protection against cardiovascular diseases, cancer, diabetes mellitus and neurological diseases) of the plants.[1,2]

In this research, total phenolic and flavonoid contents of the extracts of *Chaerophyllum bulbosum* aerial parts were determined spectrophotometrically. Also, phenolic compounds of the acetone, methanol and water extracts of *C. bulbosum* aerial parts were identified by HPLC-DAD. The total phenolic contents of the extracts ranged from 4.37±0.24 to 17.79±0.99 µg PEs/mg and decreased in the order of chloroform> hexane> water> methanol> acetone. The total flavonoid contents of the extracts ranged from 7.25±0.09 to 27.64±0.37 µg QEs/mg and decreased in the order of chloroform> acetone> water> methanol> hexane. Twenty six phenolic compounds were analyzed and seventeen of them were identified in *C. bulbosum* extracts. Rosmarinic acid was detected as major phenolic compound in *C. bulbosum* acetone extract (14.62 mg/g extract, DW), while fumaric acid was identified as main compounds in methanol and water extracts (17.43 and 18.96 mg/g extract, DW, respectively).

Acknowledgment: Authors would like to thank The Scientific and Technological Research Council of Turkey (TUBITAK-118Z610) for financial support.

References:

Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J. P. E.; Tognolini, M.; Borges, G.; Croziercorresponding, A. *Antioxid. Redox Signal.* **2013,** *18*, 1818-1892.
Nikousaleh, A.; Prakash, J. *J. Food Sci. Technol.* **2016,** *53,* 1993-2000.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Antibacterial Effects Of Streptomycin (St) Loaded Nanofibers Named PMMA/PEO/ N10-St and PMMA/PEO/N2-St

Halime ÇEVİKBAŞ[a], Seyhan ULUSOY[a], Neslihan NOHUT MAŞLAKÇI[b,c],Esin EREN[c,d], Abdullah BİÇER[e], Günseli TURGUT CİN[e], Ayşegül UYGUN ÖKSÜZ[d]

[a]*Department of Biology, Faculty of Arts and Science, Suleyman Demirel University, Isparta, 32260, Turkey*

[b]*Department of Food Processing, Gelendost Vocational School, Isparta Applied Sciences University, Isparta, 32900, Turkey*

[c]*Innovative Technologies Application and Research Center, Suleyman Demirel University, Isparta, 32260, Turkey*

[d]*Department of Chemistry, Faculty of Arts and Science, Suleyman Demirel University, Isparta, 32260, Turkey*

[e]*Department of Chemistry, Faculty of Science, Akdeniz University, Antalya, 07058, Turkey*

halimecevikbas@gmail.com

In this study, firstly fibers based on 5 wt% bis-chalcone derivatives ((2E,6E)-2,6-bis(4 nitrobenzylidene) cyclohexanone (**N10**) and (2E,6E)-2,6-bis[(thiophen-2-yl)methylene]cyclohexanone (**N2**)) were successfully prepared by electrospinning technique to increase the bioavailability of biocompatible polymethyl methacrylate (PMMA)/polyethylene oxide (PEO) fibers loaded with Streptomycin (sulfate) as an antimicrobial agent. Next, antibacterial effects of this nanofibers which were loaded with St (N10-St, N2-St, PMMA/PEO-St) and without St (N10, N2, PMMA/PEO) were examined against *Bacillus cereus* and *Escherichia coli*. Consequently, it was demonstrated that naofibers without St (N10, N2, PMMA/PEO) while didn't any antibacterial effect, nanofibers with St (N10-St, N2-St, PMMA/PEO-St) completely eradicated that *B. cereus* and *E. coli*.

2. EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

An Investigation On Effects Of Metals Ions On Formate Dehydrogenase Activity in Both Reaction Direction

Huri Bulut[a], Büşra Yüksel[b], Kübra Bozali[c], Barış Binay[d]

[a] *Bezmialem University School of Medicine, Department of Biochemsitry, Istanbu, Turkey*
[b] *Istanbul Technical University, Molecular Biology and Genetics Department, Istanbul, Turkey*
[c] *Avans University of Applied Science, Biology and Medical Laboratory Research, Netherlands*
[d] *Gebze Technical University, Department of Bioengineering, Kocaeli, Turkey*
hbulut@bezmialem.edu.tr

NAD+ - dependent Formate Dehydrogenase Enzyme (FDH) is frequently used in industrial and scientific applications. FDH is reversible enzyme that reduces the NAD+ molecule to NADH+H and produces CO2 by oxidation of formate ions and it causes CO2 reduction in the revers reaction as well (1). Transition metals W, Fe + 3 and Mo are found in the FDH structure of anaerobic microorganisms and archaea. They need these metals for their activity. However, NAD-dependent FDHs do not necessarily require these metals (2,3). Aimed to this study discover the metal dependency of NAD-dependent FDH enzymes which are from different mikroorganism gene source. In this study, FDH enzymes that have 10 different gene source (*Candida boidinii* (CbFDH), *Candida methylica* (CmFDH), *Chaetomium thermophilum* (CtFDH), *Myceliopthora thermophila* (MtFDH), *Ancylobacter aquaticus* (AaFDH), *Ceriporiopsis subvermispora* (CsFDH), *Moraxella sp.* (MsFDH), *Paracoccus sp.* (PsFDH), *Thiobacillus sp.* (TsFDH)) from different microorganisms have already cloned and expressed successfully, then effect of various metal ions on enzyme activity of forward and reverse reactions of NAD+ dependent formate dehydrogenases was investigated. In order to determine the kinetic activity of enzymes, spectrophotometric measurements were performed at 340 nm absorbance. Moreover, kinetic activity determinations were then measured for the metal ion which increased the enzymes activity most. As a result, it was found that enzyme activity improved with increasing metal ion concentration in all FDH enzymes in straight direction reactions. While enzyme activities increased with different metal ions in the range of 3-111%, the highest activity increase was caused by Fe + 3 ion in FDH enzyme from Ancylobacter aquaticus gene. Zn for CmFDH, Cu metal ion for ScFDH and CbFDH cause loss of enzyme activity in the range of 7-47%; For CtFDH, MsFDH, PsFDH and Ts FDH, both Zn and Cu ions caused loss of activity in the range of 3-82%. In forward reactions, the enzyme K_{cat} value maximum increased by 1.33-fold (5.6x103 ± 0.52 s-1) in the reaction medium containing 5 µM Mn metal ion for ScFDH; The affinity to formate was increased maximum by 1.72-fold (0.54 ± 0.61 mM) in the reaction medium containing 10 µM Mn metal ion for CtFDH. In reverse reactions, enzyme activity increased by 1-7% with different metal ions, while the maximum enzyme activity increase was caused by K ion for CsFDH. For CbFHD, PsFDH, CtFDH and MsFDH, the activity rate of 1-5% in the reaction media containing Cu ions; for TsFDH, 8% activity loss was found in Zn containing reaction medium and 9% loss was observed in reaction medium containing Cu ion. The enzyme K_{cat} value increased most by 1.13 times in the reaction medium containing 5 µM Na ions for MsFDH (0.017 ± 0.05 s-1). The affinity to hydrogen carbonate was found to be 1.18-fold (0.48 ± 0.03 mM) most in the reaction medium containing 10 µM Mo.

References:

1. Wichmann, R. 1981. Continuous enzymatic transformation in an enzyme membrane reactor with simultaneous NAD (H) regeneration, Biotechnology and Bioengineering, 23(12), 2789-2802.

2. Popov V. 0., Lamzin V. S. (1994). NAD+-dependent formate dehydrogenase, Biochem. J., 301, 625-643.

3. Tishkov, V.I., and , V.O. (2006). Protein engineering of formate dehydrogenase, Biomolecular Engineering, 23(2-3), 89-110.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Lavandula antineae Maire: An Endemic Plant With Anti-Inflammatory Properties

[a]Krimat Soumia., [a]Metidji Hafidha., [a]Ksouri Aicha., [b]Tigrine Chafia., [a]Nouasri Ahmed., [a]Dob Tahar.

[a]*Laboratory of bioactive products and biomass valorization research. ENS-kouba. , Algiers, Algeria,*
[b]*Laboratory of Ethnobotany and natural substances, ENS-kouba. , Algiers, Algeria*

krimatsoumia.2012@gmail.com

Lavandula antineae Maire (Lamiaceae) is an endemic plant in central Sahara. It is a small semi-evergreen perennial rarely distributed in Algeria, Nigeria, Chad and Sudan. Indigenous people employ this species for the treatment of chills, bruises, edema and rheumatism.

In the present study, the anti-inflammatory effect of this species was investigated for the first time using carrageenan-induced paw edema and croton oil-induced ear edema models in mice as well as the analysis of its phenolic composition by HPLC-DAD technique.

In the carrageenan induced edema test, the plant extract, at the dose of 200 mg/kg, revealed a significant anti-inflammatory activity ($P<0.05$) showing 80.74% reduction in the paw thikness comparable to that produced by the standard drug aspirin 83.44% at 4h. When the extract was applied topically at a dosage of 1 and 2 mg per ear, the percent edema reduction in treated mice was 29.45% and 74.76%, respectively. The chromatographic characterization of hydromethanolic extract by HPLC-DAD revealed the presence of various phenolic compounds: ferrulic acid, rosmarinic acid, catechin, rutin, hesperidin, naringenin, luteolin. These results demonstrate that *Lavandula antineae* Maire extract possess remarkable anti-inflammatory activity witch attributed to its phenolic content. This finding supports the folklo usage of the plant to treat various inflammatory and pain diseases.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Dynamic Mechanical Properties of Biocompatible Polymer blends of Dextran and Poly(*N*-vinyl-2-pyrrolidone)

Serap Kavlak

Hacettepe University, Faculty of Science, Department of Chemistry, 06800, Beytepe, Ankara, Turkey
skavlak@hacettepe.edu.tr

Blends of natural and synthetic polymers displays a new group of polymeric materials with better thermal/ mechanical properties and biocompatibility than those of the single polymers for the use in biomedical applications.[1] Physical/rheological/mechanical properties of polymeric materials are directly affecting the clinical efficacy. In polymeric blend system, dynamic mechanical analysis (DMA) is one of the proven techniques for investigating mechanical/rheological properties, type of transitions and intra-/intermolecular interactions using especially measurements of storage modulus (SM), loss modulus (LM) and tan d at a specific fixed frequency over a range of temperatures.

In this work, dynamic mechanical behavior of dextran (DEX) and Poly(*N*-vinyl-2-pyrrolidone) (PVP) blends was investigated as a function of composition for the further application in biomedical field. Polymeric blends were prepared at different ratios (100/0, 70/30, 50/50, 30/70 and 0/100) by solvent casting. Temperature dependence of dynamic mechanic properties of the blend system were investigated by dynamic mechanical analyzer with a novel powder-holder at a constant frequency (w = 1 Hz) using a standard temperature sweep (3 °C/min). Dynamic mechanical properties such as SM, LM, tan d, DV, CV, DF, CM *vs*. temperature and sub-glass-(as a temperature responsive parameter) and glass-transition at different a-relaxation temperatures for a DEX, PVP and blends were obtained in detail (Figure 1). Dynamic mechanical properties and compatibility in blend system can be explained by specific interactions between carbonyl and hydroxyl groups and composition.

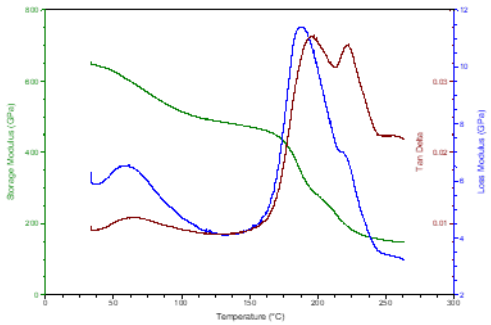


Figure 1. DMA plots of DEX:PVP (50:50) with storage modulus, loss modulus and tan d as a function of temperature.

References:

1) Sionkowska A. *Prog Polym Sci*. **2011**, *36*, 1254.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In vitro Genotoxic and Antigenotoxic effects of *Nanoliposomal Formulation of Satureja hortensis* Essential Oil Prepared by Dynamic High-Pressure Microfluidization

[a]Münevver Müge Çağal, [a]Gökçe Taner, [b]Gülengül Duman, [c]Ali Yılmaz, [c]Nagihan Nizam, [d]Fikrettin Şahin

[a]*Department of Bioengineering, Bursa Technical University,16310, Bursa, Turkey*

[b]*Faculty of Pharmacy, Yeditepe University, 34755, Istanbul, Turkey*

[c]*Department of Biotechnology, Graduate School of Natural and Applied Sciences, Bursa Technical University,16310, Bursa, Turkey*

[d]*Department of Genetics and Bioengineering, Yeditepe University,34755, Istanbul, Turkey*

munevver.cagal@btu.edu.tr

Satureja hortensis (summer savory) is an annual herb belonging to the family Lamiaceae and used as a traditional folk medicine to treat infectious diseases and disorders. Various studies have suggested that the activity of *Satureja hortensis* may relate to the strong antioxidant properties of its secondary metabolites. To improve its bioavalibility and biological activities we recently developed the nanoliposomal formulation of its essential oil by dynamic high-pressure microfluidization.

Essential oil of *S. hortensis* were prepared into the nanoliposomal drug delivery systems by microfluidization technique to provide the most homogeneous flow to produce the smallest droplet sizes. The essential oil were incorporated into nanoliposomes by ratio (2:1) using high speed homogenizer (at 20.000 rpm) and characterized. Then the essential oil of *S.hortensis* were successfully encapsulated in phospholipid based nanoliposomes. In this study our aim was to evaluate the both genotoxic and antigenotoxic effects of the nanoliposomal formulation of *S. hortensis* essential oil against mitomycin-C (MMC) in human lymphocytes. For the evaluation of genotoxicity and antigenotoxicity cytokinesis-block micronucleus (CBMN) assay in human lymphocytes were used. Human peripheral blood lymphocytes cultures were treated with 4 different concentrations (0.25, 0.5, 1, 2 µg/ml) of nanoliposomal formulation alone to assess genotoxicity and simultanously with 0,2 µg/ml MMC for antigenotoxicity. In all sets of experiments, an untreated negative control, as well as a positive control (MMC) was also run. After preparation of the slides binucleated cells surrounded by well-preserved cytoplasm were scored for the presence of micronucleus.

Micronucleus frequency was shlightly increased at all concentrations when compared with the negative control. This increase was not statistically significant at low concentrations but it has been found to be significant at higher concentration. On the other hand it has been determined in the cultures simultanously treated with MMC and the nanoliposomal formulation of *Satureja hortensis* essential oil that micronucleus frequency was decreased compared to positive control.

Keywords: *Satureja hortensis*, essential oil, nanoliposomal formulation, micronucleus, human lymphocyte culture

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Antiviral Effect of Pazar Propolis Ethanol Extract Against Herpes Simplex Virus Type 1

Merve CORA[a], Neşe KAKLIKKAYA[a], Sevgi KOLAYLI[b], Celal Kurtuluş BURUK[a], Serbülent ÜNSAL[c]

[a]*Karadeniz Technical University, Faculty of Medicine, Department of Medical Microbiology, Turkey*

[b]*Karadeniz Technical University, Faculty of Science, Department of Biochemistry, Turkey*

[c]*Karadeniz Technical University, Facult of Medicine, Department of Biostatistics and Medical Informatics, Turkey*

merve.tokdemir@yahoo.com

Aim: Propolis is a resinous substance in which the materials that the bees gather from the plants mix with the enzymes in their own saliva. Propolis is used by bees for variety of purposes of the hive. It has also been used in traditional medicine for centuries because of its pharmacological properties. Both the research studies on propolis and its use in the treatment of various diseases continuously. The aim of this study was to evaluate the antiviral effect of propolis sample obtained from Pazar (Rize, Turkey) on HSV-1.

Method: Propolis extract sample was prepared with 70% ethanol. The ethanol was removed by evaporation, and the water was removed by lyophilization from the extract, and then the dry matter was dissolved by using DMSO. The total phenolic content in the extract was determined, and the phenolic components were analyzed by HPLC-UV. The cytotoxic effect of the extract on VERO cells was determined by trypan blue staining and MTT method. The non-cytotoxic concentration of the extract to VERO cells was used in antiviral activity assays. The antiviral effect of the extract on HSV-1 was investigated by MTT, RT-PCR and plaque reduction methods. The significance of the data obtained was analyzed statistically.

Results: The total phenolic content of Pazar propolis ethanol extract was found to be 44.188 mg gallic acid equivalent / g propolis. HPLC-UV analysis revealed that the most common phenolic component in the extract was chrysin (19.28 mg extract / g sample) followed by rutin (18.03 mg extract / g sample). The extract was not cytotoxic to VERO cells at concentrations of 100 µg/mL and below. The antiviral effects of the extract on HSV-1 in MTT and plaque reduction assays were found statistically insignificant, and at 100 µg/mL concentration level in RT-PCR assay were found statistically significant ($p<0.05$).

Conclusion: Although drugs used in infections caused by HSV-1 are effective in primary infection, they may be insufficient in recurrent infection. Therefore, antiviral and vaccine development studies continue for treatment of HSV-1 infections. In this study, it has been shown that Pazar propolis can not be considered as a candidate in drug development studies for the treatment of HSV-1 infections.

This study was supported by Karadeniz Technical University Scientific Research Projects Unit (Project #: TDK-2016-5602).

Key Words: Antiviral, HPLC, HSV-1, MTT, Propolis, RT-PZR

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Borate Mineral Doping into Bone Cements in Comparison with Commercial Drug Loaded Cement in Terms of Their Anti-Bacterial Performances

Mesut KAPLAN[a], Lokman KEHRİBAR[b], Hüseyin Sina COŞKUN[c], Neslihan İDİL[d], Lokman UZUN[a,e]

[a]*Division of Bioengineering, Institute of Science, Hacettepe University, Ankara, Turkey*

[b]*Samsun Gazi State Hospital, Samsun, Turkey*

[c]*Samsun Training and Research Hospital, University of Health Science, Samsun, Turkey*

[d]*Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey*

[e]*Department of Chemistry, Faculty of Science, Hacettepe University, Ankara, Turkey*

E-mail: mesut.kaplan08@gmail.com

Polymethyl methacrylate (PMMA) is known as bone cement (BC) is one of the most ordinarily utilized items in the orthopedic procedure. İt is known as acrylic polymers that are frequently used in cement formation as fillers and fixing agents and soft tissue injuries, recurrent joint infections, chronic osteomyelitis, and open bone fractures in orthopedic and dental applications. Before applying BC in operating room conditions, the powder and the liquid portion are prepared by mixing easily. BC fits the shape of the environment in which it is located, allows for spreading of large amounts of implants and is strongly bonded. Besides, BC is also used as a drug delivery system in practice. Because of the risk of infection, BC used in joints and similar surgeries are generally preferred with antibiotic-loaded cement (vancomycin and gentamicin, tobramycin, etc.). Thanks to these cement, antibiotic are released to the environment and infections are prevented until the implant-tissue interaction. In contrast, all these positive properties of BC, the porosity of BC is very low, which limits its ability to drug release. Since the porosity is negligible, the surface drugs are generally released to the environment and sometimes this releasing can be insufficient. For this reason, patient treatment is prolonged or much more antibiotic load BC is tried to be used. Although antibiotics have a role in treating the patient, these drugs also damage the kidney and liver of the patient due to their synthetic nature. In addition, antibiotic alone is not sufficient in antibiotic-resistant infection environments and antibiotic combinations are also used. To eliminate these problems, the use of borate minerals, which are as effective as an antibiotic in the bacterial environment and which is a natural mineral, maybe the solution. Some antibacterial studies with borate minerals support this hypothesis. In this study, BC and BC containing calcium borate and sodium borate minerals were investigated in terms of antibacterial effects and the results of them were evaluated. The result showed that borate minerals can be used instead of antibiotics.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Separation of Proteins from Microbial Hyaluronic Acid Containing Fermentation Broth by Using Ion Exchange Chromatography

Özge Buldağ [a,b], Barry White[b], Dilek Kazan[a]

[a]*Marmara Üniversity, Faculty of Engineering, Department of Bioengineering,*

34722 Kadıköy/İstanbul-TURKEY

[b]*VSY Biotechnology and Pharmaceutical Industry* A.Ş. *İstanbul Tuzla* Org. *San*. Bölgesi., 3.Cad. No:3, *Tepeören*, *Tuzla*, *İstanbul-TURKEY*

ozgebuldag@hotmail.com

One of the most important macromolecule used in cosmetic and pharmacological formulations is hyaluronic acid (HA). It is a high-molecular-mass glycosaminoglycan polysaccharide that can be found in different animal tissues such as skin, cartilage, vitreous humor, synovial liquid, and bacterial cell capsule. [1] Since commercial prices of HA extracted from animal tissues/ organs is higher than that produced from animal cells, researchers and commercial companies have interested in the production of HA from microbial species to reduce the cost of HA. Among the microbial species, *Streptococcus are one of the important bacterial species used for the* production of HA [2].

Streptococcus specie are gram-positive, nutritionally fastidious, facultative anaerobes, nonmotile, non-spore forming, catalase-negative cocci that occur in pairs or chains [2]. The main problem in the production of HA processes is the removal of proteins from HA containing fermentation broth. Proteins are part of proteoglycan matrix and their level should be between 5 and 10 mg per mg HA for clinical applications. The main objective of the present work is to separate proteins from high molecular weight HA containing broth using ion exchange chromatography in combination with other purification steps [1, 5]. In order to purify HA produced from *Streptococcus sp.,* the first step is the removal of cells from fermentation broth. Tree different methods were applied to remove cells from fermentation broth: 1) direct centrifugation; 2) 1:1 dilution of broth with buffer and then centrifugation; 3) treatment of broth with SDS and centrifugation. After that, different water-miscible organic solvents as ethanol, isopropanol and acetone were used to precipitate HA from cell free broth. Among the solvents used, ethanol was found as the most effective solvent to precipitate HA. Furthermore, HA was dissolved in suitable buffer and ultrafiltration, protein precipitation, and ion exchange chromatography in different order were applied sequentially. In each purification step, amount of HA in the solution was determined by using the carbazole and turbidimetric assay and compared [3, 4]. Although the carbazole analysis was suitable to determine pure HA, it has been proven that residual glucose in the fermentation product reacts with the carbazole and does not give the accurate result. The similar results were obtained in the present work. Therefore, turbidimetric analysis was found to be suitable for the determination of HA during downstream processes. On order to determine protein content, Bradford assay [6] was used and elimination of proteins from HA containing fermentation broth was proved by electrophoresis [7].

References:

Vázquez, J.A., Pastrana, L., Piñeiro, C., Teixeira,J.A., Pérez-Martín, R.I., Amado, I.R. (2015) Production of Hyaluronic Acid by *Streptococcus zooepidemicus* on Protein Substrates Obtained from *Scyliorhinus canicula* Discards. Mar. Drugs., 13(10): 6537–6549.

Reddy, J., Karunakaran, K.T. (2013) Purification and Characterization of Hyaluronic Acid Produced by *Streptococcus Zooepidemicus* Strain 3523-7. J. BioSci. Biotech. 2(3): 173-179

Dong, Q., Zang, H., Zang,L., Liu,A., Shi, Y., Zhang,H. (2013) Rapid Determination of Hyaluronic Acid Concentration in Fermentation Broth With Near-İnfrared Spectroscopy. Journal of Innovative Optical Health Sciences. 07(06):1450012

Song, J-M., Im,J-H., Kang, J-H., Kang, D-J. (2009) A Simple Method for Hyaluronic Acid Quantification in Culture Broth. Carbohydrate Polymers, 78: 633-634

Choi,S., Choi,W., Kim, S., Lee, S-Y., Noh, I., Kim, C-W. (2014) Purification And Biocompatibility of Fermented Hyaluronic Acid for its Applications to Biomaterials , Biomaterials Research. 2014; 18: 6.

Bradford, M.A. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, 31: 426–428.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4.Nature, 227(5259): 680-685.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Influence of Some Phenolic Compounds on Aldose Reductase and Sorbitol Dehydrogenase Enzymes

Cüneyt Türkeş [a], Yeliz Demir [b], Şükrü Beydemir [c]

[a] *Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan 24100, Turkey*
[b] *Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan 75700, Turkey*
[c] *Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey*

sukrubeydemir@anadolu.edu.tr

Aldose reductase (AR, E.C. 1.1.1.21) and sorbitol dehydrogenase (SDH, EC 1.1.1.14) are enzymes of the polyol pathway, which is activated in the event of hyperglycemia that plays an essential role in the development of diabetic complications. In this study, the genes of these enzymes was cloned in E.*coli* and purified. The purification of enzymes was performed by Ni-NTA affinity chromatography. The molecular masses of SUMO-hAR and SUMO-hSDH fusion proteins were determined as 47.54 and 49.04 kDa, respectively. Then, the effects of some phenolic compounds on hAR and hSDH activities were examined. These compounds include 4-methylcatechol, danshensu sodium, esculin hydrate, morin hydrate, naringin, phloridzin dihydrate, prunetin, schizandrin, and vanillic acid showed the potent inhibitory effects for hAR and hSDH (K_i*s* in the ranging from 6.35 to 77.22 mM and 1.99 to 36.75 mM, respectively).

Acknowledgments

This work was supported by the Research Fund of Erzincan Binali Yıldırım University (grant number FBA -2017-501).

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Inhibitory Properties of Bis-thiomethylcyclohexanone Derivatives on Aldose Reductase Enzyme Activity

Cüneyt Türkeş [a], Yeliz Demir [b], Abdullah Biçer [c], Şükrü Beydemir [d], Günseli Turgut Cin [c], Mehmet Serdar Gültekin [e,f]

[a] *Department of Biochemistry, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan 24100, Turkey*
[b] *Department of Pharmacy Services, Nihat Delibalta Göle Vocational High School, Ardahan University, Ardahan 75700, Turkey*
[c] *Department of Chemistry, Faculty of Arts and Sciences, Akdeniz University, Antalya 07058, Turkey*
[d] *Department of Biochemistry, Faculty of Pharmacy, Anadolu University, Eskişehir 26470, Turkey*
[e] *Department of Chemistry, Faculty of Arts and Sciences, Atatürk University, Erzurum 25240, Turkey*
[f] *Faculty of Pharmacy, Ağrı İbrahim Çeçen University, Ağrı 04100, Turkey*

sukrubeydemir@anadolu.edu.tr

The synthesis of sulfur-containing pharmaceutical and natural products is a significant methodology. Bis-sulfide and sulfide compounds have crucial biological activities in pharmaceutical and applications. Aldose reductase (AR) belongs to NADPH-dependent oxidoreductases. It reduces glucose to sorbitol. AR inhibition is important to prevent diabetic complications such as nephropathy, neuropathy, cataract, and retinopathy. In this study, it was evaluated the effect of bis-thiomethylcyclohexanone derivatives on AR enzyme activity. The IC_{50} values of these compounds were found in the range of 0.91± 0.01–1.77 ± 0.04 µM for AR. 2,6-bis((3-nitrophenyl)(phenylthio)methyl)cyclohexanone (3c) showed the best inhibition effect according to the IC_{50} values. As a result of this study, bis-thiomethylcyclohexanone compounds record novel AR enzyme inhibitor and may have promising anti-diabetic drug potential.

Acknowledgments

This work was supported by the Scientific Research Projects Coordination Unit of Akdeniz University (Project Number FDK-2016-1541), and the Research Fund of Erzincan Binali Yıldırım University (grant number FBA-2017-501).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Development Of A Novel Graphene/Azobenzene-Perylene Diimide Derivative Modified Electrochemical Dopamine Sensor Through A Chemometric Approach

Tuğba ÖREN VAROL[a], Benay PERK[a], Okan AVCI[a], Oğuz AKPOLAT[a], Özgül HAKLI[a], Chenming XUE[b], Quan LI[b], Ülkü ANIK[a]

[a] *Muğla Sıtkı Koçman University, Faculty of Science, Chemistry Department, Kötekli-Muğla, 48000, Turkey*

[b] *Liquid Crystal Institute, Kent State University, Kent, OH 44242, United States*

tugbaoren@mu.edu.tr

Dopamine is a vital biomolecule and a neurotransmitter for the regulation of central nervous system. Abnormal dopamine concentrations in biological fluids are considered to be associated with Parkinson's disease, schizophrenia, attention deficit hyperactivity disorder and epilepsy.[1,2] Thus, diagnosis of these mentioned diseases requires practical, accurate and reliable detection of dopamine.

The aim of this study is the development of a practical and sensitive electrochemical dopamine sensor by using a novel azobenzene-perylenediimide derivative and graphene. Experimental parameters (azobenzene-perylenediimide derivative and graphene amount, supporting electrolyte pH and scan rate) were optimized by using full factorial experimental design. After the optimization of experimental parameters, analytical characteristics of the developed sensor were examined and calibration graph was plotted. A linear range was obtained in the concentration range of 5-100 µM with a detection limit of 0.26 µM (n=3). The repeatability was evaluated in terms of relative standard deviation and calculated for 25 µM dopamine as 2.1% (n=3). Interfering effects of ascorbic acid and uric acid on dopamine oxidation peak current were evaluated and the performance of the developed sensor in analytical applications was tested in dopamine hydrochloride injection samples with satisfactory results. According to the obtained data, graphene/azobenzene-perylene diimide derivative modified electrochemical sensor has a potential to be used for the dopamine detection in medical applications.

References:

Ören, T.; Birel, Ö.; Anık, Ü. *Anal. Lett.* **2018**, *51*, 1680.
Haldorai, Y.; Vilian, A. E.; Rethinasabapathy, M.; Huh, Y. S.; Han, Y.-K. *Sens. Actuators, B* **2017**, *247*, 61.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Antiradical and Anticholinesterase Activities of the Four *Trametes* Species

Ebru Deveci[a,b,*], Fatih Çayan[c], Gülsen Tel-Çayan[c], Mehmet Emin Duru[b]

[a]*Muğla Sıtkı Kocman University, Faculty of Science, Department of Chemistry, Muğla, TURKEY*

[b]*Konya Technical University, Technical Sciences Vocational School, Chemistry and Chemical Processing Technology Department, Konya, TURKEY*

[c]*Muğla Sıtkı Kocman University, Muğla Vocational School, Department of Chemistry and Chemical Processing Technologies, Muğla, TURKEY*

**Corresponding Author: ezengin@mu.edu.tr*

Trametes species, a member of the Polyporaceae family, grow especially in warm climates and there are about 60 *Trametes* species in the world. *Trametes* species are considered to be one of the most important medicinal mushroom species in the world. Most actively studied in the past two decades mushroom *T. versicolor* (Turkey tail) has been identified as the natural source of polysaccharides krestin (PSK) and polysaccharide peptide (PSP).[1]

Antiradical and anticholinesterase activities of the hexane, acetone and methanol extracts of *Trametes bicolor, T. pubescens, T. suaveolens* and *T. versicolor* were evaluated in this study. Antiradical activity was tested by using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals and anticholinesterase activity was determined by Ellman method. According to obtained results, in $ABTS^{\bullet+}$ assay, the acetone (87.98±1.05 %) and methanol extracts (89.95±0.38 %) of *T. pubescens* and the methanol extract (88.47±1.72 %) of *T. versicolor* were found as the best scavenger. In addition, these extracts showed higher activity than standards (BHA and α-tocopherol). The acetone extract (85.68±0.28 %) of *T. pubescens* and the methanol extract (73.82±0.71 %) of *T. versicolor* were found as the best scavenger in $DPPH^{\bullet}$ assay. Among the studied extracts, the hexane extract (82.38±1.09 %) of *T. pubescens* was found as the most active against AChE, also showed higher activity as galantamine (80.41±0.98 %). The hexane (73.64±0.15 %) and acetone extracts (74.88±0.55) of *T. pubescens* indicated the best inhibitory activity against BChE.

References:

Hobbs, C. R. *Int. J. Med. Mushrooms.* **2004,** *6,* 195-218.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Covalent immobilization of cellulase on epoxy methacrylate and epoxy/butly methacrylate resins

Güzide Yücebilgiç, Fatma Nükte Çölkesen
Cukurova University, Science and Letters Faculty, Chemistry Department, Adana, Turkey
Email address:gybilgic@cu.edu.tr

Cellulases catalyze hydrolysis of cellulose and they have numerous practical applications in many fields of industry and agriculture. In view of the broad application of cellullases, it is necessary to develop more stable cellulase preparation to enable the aforementioned processes to be carried out more efficiently.[1] Enzyme immobilization techniques have been successfully applied in order to improve the properties (stability, activity and selectivity) of different enzymes.[2] Epoxy groups are stable at neutral pH, but they easily react with amine, hydroxyl, and thiol groups on the surface of enzymes and form a strong linkage with minimum changes in the structure of enzymes. Therefore, epoxy-activated resins are almost ideal matrices to perform easy immobilization of enzymes.

In this study, cellulase was immobilized on epoxy methacrylate and epoxy/butly methacrylate resins by covalently and the obtained immobilized enzymes were characterized. The free enzyme showed its optimum pH at 5.5, while the both immobilized enzymes showed at 6.5. The optimum temperature of all cellulase preparation had a maximum activity at 50 °C.

References:

Zdarta,cJ.; Jędrzak, A.; Klapiszewski, Ł.; Jesionowski, T. *Catalysts* **2017**, 374.
Mateo, C.; Palomo, J.M.; Fernandez-Lorente, G.; Guisan, J.M.; Fernandez-Lafuente, R. *Enzyme Microb. Technol.* **2007,** 1451.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Carbonic Anhydrase Inhibitory Activities of Herbal Teas and Their Phenolic Composition Analyzed by HPLC-DAD-UV

Ayça Aktaş Karaçelik [a], Semra Alkan Türkuçar[b], Murat Küçük[c]

[a]*Department of Food Processing, Espiye Vocational School, Giresun University, Giresun, Turkey*

[b] *Alanya Alaaddin Keykubat University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Alanya, Antalya, Turkey*

[c]*Department of Chemistry, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey*

e-mail: aycaaktas84@hotmail.com

Since the earliest times, herbal teas have been used both as basic nutrients and as drug sources prepared by infusion of plants. Studies over the last few years have shown that bioactive compounds in plants also exhibit carbonic anhydrase (CA) inhibition profile.[1, 2] Thus, the effects of aqueous extracts of sage, linden, rosehip, chamomile, clove, echinacea, cinnamon and mint on bovine carbonic anhydrase (BCA) and human carbonic anhydrase isoenzymes hCAI and hCAII, purified by affinity column chromatography, were investigated. For this purpose, measurements of the esterase activity of the enzyme were performed at five different concentrations of each herbal tea extract. IC_{50} values, the inhibitor concentration causing 50% inhibition, were calculated by drawing % activity vs inhibitor concentration (mg/mL) plots. In addition, an HPLC -DAD-UV method was developed to identify and quantify phenolic compounds in herbal teas with using 14 standard phenolics.

In herbal teas ten phenolic compounds (gallic acid, protocatechuic acid, protocatecaldehyde, p-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringaldehyde, ferulic acid) were detected. Protocatechuic acid was the most frequently observed phenolic. IC_{50} values of the samples were seen in the range of 0.005-1.037 mg/mL. According to the results, clove ($IC_{50\,(BCA)}$: 0.005 mg/mL, $IC_{50(hCAI)}$: 0.076 mg/mL, $IC_{50\,(hCAII)}$: 0.052 mg/mL), cinnamon ($IC_{50(BCA)}$: 0.008 mg/mL, $IC_{50(hCAI)}$: 0.097 mg/mL, $IC_{50(hCAII)}$: 0.017 mg/mL) and mint ($IC_{50(BCA)}$: 0.152 mg/mL, $IC_{50(hCAI)}$: 0.056 mg/mL, $IC_{50(hCAII)}$: 0.030 mg/mL) showed high inhibitory effect on BCA, hCAI and hCAII isoenzymes. Echinacea ($IC_{50(BCA)}$: 0.921 mg/mL, $IC_{50(hCAII)}$: 1.014 mg/mL) has a low inhibition effect on BCA and hCAII isoenzymes, while rosehip ($IC_{50\,(hCAI)}$: 0.671 mg/mL) has a low effect on hCAI isoenzyme.

We believe that these findings will contribute to the development of new chemotypes with improved drug-like properties in the treatment of diseases related to CA enzyme to support the wider evaluation of natural products.

*This study was supported by 2211-C Priority Areas Domestic PhD Scholarship Program of TUBITAK-BIDEB.

References:

Şentürk, M.; Gülçin, i.; Daştan, A.; Küfrevioğlu, Ö. İ; Supuran, C. T.,. *Bioorg. Med. Chem*. **2009**, 17.

Sarikaya, S. B. O.; Gülçin, I.; Supuran, C. T.,. *Chem Biol Drug Des*. **2010**, 75, 5.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Characterization of Lactate Dehydrogenase Immobilized onto Mesoporous Silica

Dilek Alagöz [a] , Deniz Yildirim[b], Ali Toprak[c] , Nazlı Ece Varan[d] S.Seyhan Tükel[d]
[a] *Vacational School of Imamoglu , University of Cukurova, Adana, TURKEY*
[b] *Vacational School of Ceyhan , University of Cukurova, Adana, TURKEY*
[c] *Vacational School of Acıgöl , University of Nevşehir, Nevşehir, TURKEY*
[d] *Science and Letters Faculty , University of Cukurova, Adana, TURKEY*
Corresponding author: dalagoz@cu.edu.tr / alagozdilek@yahoo.com

Lactate dehydrogenase (LDH) catalyzes the conversion of lactate to pyruvate in the presence of NAD^+ and the NADH produced in the enzymatic reaction can be measured, according to the following reactions [1]:

$$LDH + Lactate + NAD \longrightarrow pyruvate + NADH + H^+$$
$$NADH \longrightarrow NAD + H + 2e$$

Enzyme immobilization onto insoluble support is a desired biological procedure because of its possible applications in product purification and catalyst recycling [2](Jia et al., 2003). Furthermore, immobilization provides many advantages such as: 1) enhanced stability, 2) easy separation of product from reaction mixture, 3) possible modulation of the catalytic properties, 4) easier prevention of microbial growth[3] .

In this study, lactate dehydrogenase (LDH) from Porcine was covalently immobilized onto mesoporous silica. The activities of free and immobilized LDH were spectrophotometrically measured at 340 nm using lactate and NAD+ as substrate. The maximum pH and temperature of free and immobilized LDH were investigated and determined as 10.0 and 40 °C, respectively for the free and 9.0 and 35 °C, respectively for the immobilized LDH. The thermal stability of free and immobilized LDH were studied by measuring the residual activities of LDH preparations at specific time intervals. The result showed that the thermal stability of free LDH was enhanced upon immobilization.

Acknowledgements: This project was supported by Scientific Research Projects Unit of Cukurova University of with the project number of FBA-2018-11026.

References

Sass C.; Briand,M.; Benslimane S.; Renaud M.; Briand Y.; J. BioI. Chem. **1989,** (264) 4076-4081.
Jia, H.; Guangyu, Z.; W Ang. P .;Biotech. Bioeng., **2003**, 84: 407- 413.
Bornscheuer, U.T., Angew. Chem. Int., **2003,** 42: 3336-3337.

2.EBAT
EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Immobilization and stabilization of a NADP$^+$-dependent multimeric alcohol dehydrogenase from *E.coli*

Nazlı Ece Varan[a], Dilek Alagöz[b], Seyhan Tükel[a], Deniz Yıldırım[c]

[a]*Cukurova University, Science and Letters Faculty, Chemistry Department, Adana, Turkey*

[b]*Cukurova University, Imamoğlu Vocational School, Adana, Turkey*

[b]*Cukurova University, Ceyhan Vocational School, Adana, Turkey*

Email address:ece.varan@gmail.com

Alcohol dehydrogenases (ADH) have been widely used as a biocatalyst in synthetic chemistry since they catalyze chemeo-, regio-, enantio-selective oxidation and reduction reactions. However, their industrial uses are limited by poor temperature and pH stability. In recent years, the stabilization of ADHs has been achieved using different techniques such as protein engineering, chemical modification and immobilization.[1] Enzyme immobilization techniques have been successfully applied in order to improve the properties (stability, activity and selectivity) of different enzymes.[2] Entrapment of enzymes in polymeric matrices is an easy technique to prepare recyclable, stable, and cost-effective immobilize biocatalysts for industrial application. Entrapment of enzyme in polyvinyl alcohol (PVA) hydrogel offers several advantages, such as low matrix cost, inexpensive and simple gel preparation, low diffusion limits, excellent mechanical stability.[3]

In this study, a NADP$^+$-dependent multimeric alcohol dehydrogenase was immobilized in PVA hydrogel and characterized in terms of optimal pH and temperature. The both free and entrapped ADHs had maximum activity at pH 8.0 and 30 °C. The entrapped ADH showed higher thermal stability than that of the free ADH at 30 °C. These result show that entrapment of ADH in PVA leads active and stable enzyme preparation.

References:

Shinde, P; Musameh, M.; Gao, Y.; Robinson, A.J.; Kyratzis, I. *Biotechnol. Rep.* **2018**, e00260.
Mateo, C.; Palomo, J.M.; Fernandez-Lorente, G.; Guisan, J.M.; Fernandez-Lafuente, R. *Enzyme Mirob. Technol.* **2007,** 1451.
Yildirim, D.; Alagöz, D.; Toprak, A.; Tükel, S.; Fernandez-Lafuente, R. *Process Biochem.* **2019,** 97.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Decolorization of Reactive Yellow-15 Textile Dye by Immobilized Laccase onto Sunflower Stalks

Didem TUNCAY[a], Hülya YAĞAR[b]

[a]*Trakya University, Edirne Vocational College of Technical Sciences-Edirne*

[b]*Trakya University, Faculty of Science, Department of Chemistry-Edirne*

didemtuncay@trakya.edu.tr

The advancing biotechnological methods and environmental awareness have enhanced their alternative areas of usage as biofuel and material of construction as well as the traditional utilization of agricultural wastes. Recycling of agricultural wastes provides both the solution to environmental problems and has a place in economic significance. Trakya Region, which is one of the important areas for agricultural activity of our country, is under serious threat due to industrial water pollution in Ergene basin. This study suggests a model on the utilization of regional agricultural wastes (rice husk, sunflower husk, stalk, etc.) to eliminate industrial water pollution in the region.

Agricultural wastes can be used as support materials in enzyme immobilization due to their lignocellulosic structure. In this study, laccase was immobilized onto sunflower stalk and the effect of immobilized laccase for decolorization of textile wastewater was investigated.

Laccases (EC. 1.10.3.2) are a member of multi-copper containing enzymes that catalyze the oxidation of many aromatic compounds. The reactions catalyzed by laccase have broad range of potential for eco-friendly applications since they only use molecular oxygen as co-substrate and release only water as byproduct during reaction. They catalyze the radical breakdown of the chromophore groups of textile dyes, thereby removing the color of the dyestuffs.

The dried and grind sunflower stalks were delignified to prepare as immobilization support. The laccase isolated from *Boletus edulis* mushroom was concentrated by three phase partitioning and dialysis methods and then, immobilized on delignified sunflower stalks by adsorption method. The immobilization yield was determined as 93%. For decolorization process, Reactive Yellow-15 (RY-15) dye solution (200 mg/L) prepared in 0,1 M sodium-citrate buffer (pH 3,0) was incubated with 100 mg of immobilized laccase at 50 °C for 1 hour. The decolorization rate of RY-15 dye of immobilized system was calculated as 87.6%.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Formation of a Curcumin-Copper Conjugate and the Evaluation of its Antimicrobial Activity

Ebru Köleoğlu[a], Tayfun Acar[b], Serap Derman[b], Berna Sarıyar Akbulut[a]

[a] *Bioengineering Department, Marmara University, Istanbul, Turkey*
[b] *Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, Istanbul, Turkey*

ebru_koleoglu@hotmail.com

Growing public interest in traditional medicine, particularly plant-based medicine, has leds comprehensive research on the potential of natural substances. Among plant-derived products, curcumin is a polyphenol derivative found in turmeric (*Curcuma Longa L.*) with anti-inflammatory, antioxidant, antimicrobial, wound healing, and biofilm inhibition properties. Despite its potential, its use is often limited due to its low water solubility, poor bioavailability that lead to poor absorption, and instability in neutral and basic pH. The current work investigates the conjugation of curcumin to copper to improve its properties. To this end, a mononuclear curcumin-copper(II) complex (1:1 mol) was synthesized with copper(II) following the protocols of Barik et al. (2005). The physicochemical properties of the complexes were characterized by UV absorption and FTIR spectroscopy. Then the biological activity of the synthesized complex was evaluated by comparing it with the activity of curcumin and copper(II) alone and with the activity of their mixture in *Escherichia coli* cell.[2,3] Results have indicated that curcumin showed chelation with copper(II), as confirmed by the FTIR (shifting of stretching vibrations of ν(C=C) and ν(C=O)), and UV spectroscopy (deconvoluted with absorption band at 432–456 nm) when copper(II) acetate was used. It was not possible to form a complex with copper sulfate(II). The minimum inhibitory concentration of curcumin and copper (II) were 128 μg ml^{-1} and 256 μg ml^{-1}, respectively, while the minimum inhibitory concentration of the formed complex was 64 μg ml^{-1}. Furthermore, checkerboard assay showed that the combinations of curcumin and copper(II) revealed synergistic activity. Our findings showed that the minimum inhibitory concentration of the curcumin-copper(II) complex and the results of checkerboard assay are consistent. With these results, we believe that curcumin-copper(II) acetate complex could improve the properties curcumin and these results may provide useful information for the investigation of curcumin-copper(II) complex in therapeutic applications.

References:

Barik A, Mishra B, Shen L, vd. Free Radical Bio. Med. **2005**, 39, 811.
Orhan G, Bayram A, Zer Y, Balci I. J ClinMicrobiol **2005**, 43: 140-143.
Wiegand I, Hilpert K, Hancock RE. Nature Protocols, **2008,** 3(2), 163-175.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Preparing Chitosan/Wallostonite Scaffolds and Their Compatibility Assessments

Ece Karakaya[a], İpek Ertuğrul[b], Ayça Gürbüz[b], Önder Taşar[b], Kübra Kaya[b], Emel Emregül[a], Sedat Odabaş[b]

a Ankara University, Faculty of Science, Department of Chemistry and PMBR Lab. Ankara TURKEY

b Ankara University, Faculty of Science, Department of Chemistry and BteLAB. Ankara TURKEY

Corresponding author: Ece KARAKAYA , karakaya.ece@hotmail.com

Tissue Engineering is a key principle that aims to repair and regenerate the tissues using appropriate cells, growth factors of generally with scaffolds. There are several natural and synthetic materials reported for the preparing a scaffold. Moreover, hybrid scaffolds that contain many components are crucial for particular tissues such as bone and cartilage. Chitosan is a biopolymer that induces osteogenesis and Calcium Silicate may act as an osteoconductive biomaterial. Within this study, Chitosan/Wallostonite Scaffolds were prepared and chemically characterized by FTIR and XRD also morphologically characterized by SEM. Moreover, their biocompatibility was assessed by MTT assay using osteoblast cells and hemocompatibility by hemolysis evaluation. Results show that these hybrid scaffolds have proper integrity in their structure. Cells are well integrated with the scaffolds where their pores are suitable for cell proliferation and growth. As a conclusion, these Chitosan/Wallostonite hybrid scaffolds can be a good candidate for hard tissue engineering.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effect of Water-Soluble Calix[n]arenes on the Superactivity of *Candida rugosa* Lipase

Elif OZYILMAZ[a], Filiz ESKI[b]

[a]*Department of Biochemistry, Selcuk University, 42075 Konya, Turkey*
[b]*Department of Chemistry, Selcuk University, 42075 Konya, Turkey*
eyilmaz80@gmail.com

Calix[n]arenes are supramolecular compounds able to form host-guest inclusion complexes with ions, small organic molecules, and small moieties of larger molecules.[1] In recent years it has been observed that calixarenes are carrier of amino acid, protein, collagens and enzymes.[2] The active site of flap/lid of lipase (CRL) has 31 amino acids, mainly hydrophilic on its external face and hydrophobic on the internal side, directed towards the active site is the hydrophilic surface of the molecule in the open form and the inactive site is the hydrophobic surface of the molecule in the closed form. [3-5] Calix[n]arenes interact with amino acids on the lid or with strong hydrogen bonds that leads to open up the lid and thus the activity of the enzyme is maintained for a long time.[2] In addition, the calixarenes interact with different functional groups of the enzyme to preserve the conformation.[6] In this regard, in this study water soluble calixarene derivatives were first time used as a surfactant to see effect on the hydrolytic activity of *Candida rugosa* lipase. The hydrolysis of p-nitrophenyl palmitate as probe reaction was investigated and the dependence of reaction rates on surfactant concentrations and pH was studied.

References :

1) Li, C.P.; Tan S.; Ye, H.; Cao, J., Zhao, H. *Food Chem*. **2019**, *283*, 191–198.
2) Yilmaz M., Sayin S., in: P. Neri, J. Sessler, M.X. Wang (Eds.), Calixarenes in Organo and Biomimetic Catalysis, Calixarenes and Beyond, Springer, 2016.
3) Lotti, M.; Tramontano, A;, Longhi, S.; Fusetti, F.; Brocca, S.; Pizzi, E.; Alberghina, L. *Protein Eng.* **1994**, *7*, 531–535.
4) Hernández, J.H.; del Pozo, J.T.; Canelo, P.M.; Puebla, A.R.; Tejo, S.P.; Marcos, J.S. *Arch. Bronconeumol.* **2004,** *40*, 304–310.
5) Gabriele, F.; Spreti, N.; Giacco, T.D.; Germani, R; Tiecco, M.; *Langmuir.* **2018**, 34, 11510-11517.
6) Ozyilmaz, E.; Bayrakci, M.; Yilmaz, M. *Bioorg. Chem.* **2016**, 65, 1–8.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Identification of *AKT1*/*CTNNB1* Mutations Conferring Cetuximab and Chemotherapeutic Drugs Resistance in Colorectal Cancer Treatment

Gozde Hasbal, Pinar Aksoy-Sagirli, Ayse Can

Department of Biochemistry, Faculty of Pharmacy, Istanbul University, Beyazıt, Istanbul, Turkey.

gzdehasbal@hotmail.com/gozde.hasbal@istanbul.edu.tr

In anti-cancer therapy, the effectiveness of therapeutics is limited by mutations causing drug resistance.[1] *KRAS* mutations are the only determinant for cetuximab resistance in colorectal cancer (CRC) patients.[2] Therefore, it is very important to determine new predictive mutations in CRC treatment.[3] In this study, the association of *AKT1*/*CTNNB1* mutations with the drug resistance against cetuximab and some chemotherapeutics used in the CRC treatment were investigated *in vitro* by using site-directed mutagenesis, transfection, Western Blot methods, and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell growth inhibition assay. The cetuximab resistance was higher in the presence of *AKT1* E17K, E49K, and L52R mutations and, *CTNNB1* T41A, S45F, and S33P mutations compared to that of the WT. *AKT1*/*CTNNB1* mutations were also determined to be associated with oxaliplatin, irinotecan, SN-38, and 5-fluorouracil resistance. Furthermore, mutant cell viability in oxaliplatin treatment was more effectively inhibited than that of the other drugs. These findings provide evidence that CRC patients carrying *AKT1/CTNNB1* mutations may have resistance to cetuximab and frequently used chemotherapeutics, and these mutations may serve as an important predictive biomarker which responsible for drug resistance.

Kaynaklar

1) Longley, D.B.; Johnston, P.G. *J. Pathol.* **2005**, 205, 275.
2) Jančík, S.; Drábek, J.; Radzioch, D.; Hajduch, M. *BioMed. Res. Intl.* **2010**, Article ID 150960.
3) Arqués, O.; Chicote, I.; Puig, I.; Tenbaum, S.P.; Argilés, G.; Dienstmann, R.; Fernandez, N.; Caratu, G.; Matito, J.; Silberschmidt, D.; Rodon, J.; Landolfi, S.; Prat, A.; Espin, E.; Charco, R.; Nuciforo, P.; Vivancos, A.; Shao, W.; Tabernero, J.; Palmer, H.G. *Clin. Cancer Res.* **2016**, 22, 644.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The cytotoxic effects of *Ornithogalum oligophyllum* and *Ornithogalum umbellatum extracts*

Gulsum Altiparmak-Ulbegi[a], Gozde Hasbal[a], Pinar Aksoy-Sagirli[a], Tugba Yilmaz-Ozden[a], Mine Kocyigit[b], Refiye Yanardag[c], Ayse Can[a]

[a]*Istanbul University, Faculty of Pharmacy, Department of Biochemistry, Istanbul, Turkey.*

[b]*Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey.*

[c]*Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Biochemistry, Istanbul, Turkey.*

glsm.altiparmak@gmail.com

Ornithogalum (Asparagaceae) is a genus with more than 100 species are used in traditionally in many countries, also consumed in Turkey both as food and for therapeutic purposes. In Turkey, the genus is represented by 36 species and its regional name is "tükrük otu". This study aimed to investigate the cytotoxic effects of *Ornithogalum oligophyllum* and *Ornithogalum umbellatum*. In this context, methanol and water extracts were prepared from the aerial parts and bulbs of the plants using the Soxhlet apparatus. The cytotoxic activities of the extracts on prostate (PC-3), lung (A549), endometrium (ECC-1) cancer cell lines and human umbilical vein/vascular endothelium (HUVEC) control cell line were determined using [3-(4,5-dimethylthiazol-2-yl)-5-(3- carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) cell viability assay. According to the our findings, water extracts of *O. oligophyllum* and *O. umbellatum* had no remarkable cytotoxicity. However, methanol extracts of *O. oligophyllum* and *O. umbellatum* showed the cytotoxic effect on ECC- 1 cell line. In addition to that, methanol extract of *O. umbellatum* aerial parts exhibited cytotoxic activity on PC-3 cancer cell line. In summary, methanolic extracts from *O. oligophyllum* and *O. umbellatum* showed medium cytotoxic activity against the endometrium and prostate cancer cell line. These findings might be a database for future studies.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

A Biodegradable Polyurethane-Dextran Bioink for Potential 3D Bioprinting

Merve Goksin KARAASLAN[1], Fatih INCI[1],* and Utkan DEMIRCI[1],*

[1]*Bio-Acoustic MEMS in Medicine (BAMM) Laboratory, Canary Center at Stanford for Cancer Early Detection, Department of Radiology, Stanford School of Medicine,* Stanford University, *Palo Alto USA*

**co-corresponding authors: Fatih INCI (finci@stanford.edu) and Utkan DEMIRCI(utkan@stanford.edu)*

mgkaraaslan@gmail.com

Three-dimensional bioprinting technology is a powerful tool that produces promising solutions in the field of tissue engineering and regenerative medicine. However, cell printing systems utilizing synthetic and biological inks still constitute some challenges regarding biocompatibility and viability in tissue engineering applications. Accordingly, current bioinks need further improvements in their rheological properties, structural integrity, and biocompatibility features. Polyurethane structures have some unique properties with versatile mechanical characteristics that hold a great potential as bioink1.

In this study, we designed a novel waterborne polyurethanes that reacted with 4,4′-methylenebis (cyclohexyl isocyanate), polyethylene glycol (PEG400), dextran (Mw:100000 g/mol). Different molar ratios of PEG400 / Dextran / 2,2-bis(hydroxymethyl) propionic acid (DMPA)/ Ethylenediamine (EDA) were examined. The structure of final product was examined with infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), differential thermal analysis (DTA). Its biocompatibility and biodegradability properties were assessed with in the condition of physiological pH. At the end of 3 weeks, the biodegradability of the polymer was identified at range of 19.71 ± 2.65 % - 79.17 ± 5.65 %. The indirect cytotoxicities of the synthesized WPUs structures were determined on NIH-3T3 fibroblast. In cell cullture experiment, all polyurethanes showed cell viability at range of 73.33±9.53 - 88.63±7.20% according to in vitro biocompatibilty test. We observed that the presented waterborne polyurethane materials containing dextran hold great impact as a novel bioink candidate for 3D cell-laden constructs.

References

1) Hung, K. C.; Tseng, C.S. ; Hsu, S.H. Advances in Polyurethane Biomaterials. **2016**, 149-170.

-This study was supported by grants from TUBITAK (2219).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Inhibitory Effects of Lactoferrin on Biofilm Formation of *Pseudomonas aeruginosa*

Seyhan ULUSOY, Halime ÇEVİKBAŞ
Süleyman Demirel University,Department of Biology, Isparta
seyhanulusoy@sdu.edu.tr

Lactoferrin (LF) is an iron-binding antimicrobial protein present in saliva, tears, and milk; and it is present in neutrophil granules[1]. *Pseudomonas aeruginosa (P. aeruginosa)* is an opportunistic pathogen, and has been identified as a principal biofilm-forming opportunistic pathogen in chronic wounds[2]. The aim of the current study was to assess the in vitro effect of LF on biofilm formation, of *P. aeruginosa.*

We evaluated the in vitro effects of lactoferrin on biofilm formation by *P. aeruginosa* PA01 using a crystal violet assay. Lactoferrin significantly inhibited biofilm formation in *P. aeruginosa* (90% inhibition). These results demonstrate the antibiofilm activity of LF against *P. aeruginosa* and the potential usefulness of LF for the prevention Pseudomonal diseases.

References:

Levay, P. F., and M. Viljoen. 1995. Lactoferrin: a general review.Haematologica 80:252-267

K. Gjodsbol, J.J. Christensen, T.Karlsmark, B. Jorgensen, B.M. Klein, K.A. Krogfelt, 2006. Multiple bacterial species reside in chronic wounds: a longitudinal study.Int Wound J, 3, pp. 225-231

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Mitochondrial DNA-Induced Problems in Plasma During Extracorporeal Circulation: Comparison of Centrifugal and Roller Pumps

Oğuzhan DURMAZ

Ankara University Faculty of Medicine, Cardiovascular Surgery, Perfusion Department

durmazogz@gmail.com

Used in cardiovascular surgery; Extracorporeal circulation (ECC) systems; cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation (ECMO) may cause inflammatory responses. Pumps used during ECC cause mechanical cell damage, resulting in increased mitochondrial DNA (mtDNA) in plasma. In circulation, mtDNA fragments trigger a series of molecular responses that cause inflammatory responses. Some studies on this were examined. The positive correlation between mtDNA and inflammatory cytokines after CPB suggests that mtDNA may play an important role in inflammation. To reduce mtDNA and inflammation, more molecular studies should be performed by comparing different systems and pumps (roller and centrifugal). Parameters such as flow, cannulation type, CPB / ECMO time, different age groups should be researched separately. To reduce the mechanical cell damage, more clinical datas are needed to determine the ideal strategy ECC.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Investigation of Intermolecular Interactions between Functional Monomers and Template Molecules for the Synthesis of Polyamine Imprinted Polymers

Pınar Çakır Hatır[a], Elif Çepni Yüzbaşıoğlu[b], Oğuz Yücel[c], Nur Muhammed Mustafaoğlu[d], Şeyma Türker[a]

[a]*Department of Biomedical Engineering, Istanbul Arel University, Turkey*
[b]*Department of Molecular Biology and Genetics, Istanbul University, Turkey*
[c]*Department of Chemical Engineering, Istanbul University Cerrahpaşa, Turkey*
[d]*Department of Molecular Biology and Genetics, Istanbul Arel University, Turkey*
turkerseyma11@gmail.com

Polyamines (putrescine, spermidine, and spermine), one of the important raw materials for cosmetic industry, are organic compounds containing two or more amino groups. Polyamines, found in rice bran and used as chicken feed, have been used as raw materials in cosmetic industry for many years especially because of their hair and nail growth promotion and hair repair properties, anti-allergenic, anti-glycation and anti-aging effects.[1] It is therefore very important to extract polyamines in pure form from their sources. Molecularly imprinted polymers (MIP), which are synthetic polymers tailored to recognize specific molecules, are used in many applications such as separation, drug delivery systems and sensor applications.[2] One of the most important parameters of MIP synthesis is the interactions between functional monomers and the target molecule. It is well known that the stronger this interaction, the higher their molecular recognition efficiency. The purpose of the proposed study is to investigate the molecular interactions between polyamines and functional monomers before synthesizing polyamine imprinted polymers. Interactions between putrescine, spermine, and spermidine and 4 vinyl pyridine (4VP), methacrylic acid (MAA) and acrylamide (AAm) were investigated by UV/Vis and FTIR spectroscopy in different solvents. As a result of the study, various interactions between polyamines and functional monomers were determined. The interactions between 4VP and polyamines were found to be hydrophobic in aqueous phases, additionally, it was observed that polyamine interactions with AAm and MAA were stronger in nonpolar solvents.

Acknowledgmends

This research was supported by the financial support from TUBITAK, Scientific and Technological Research Council of Turkey, Project No: 116Z880. The authors thank Hasan Çubuk for his support in the experiments.

References

Yatin, M. *J. Cell Mol. Biol*. **2002**, *1*, 57.

Çakir, P.; Cutivet, A.; Resmini, M.; Bui, B. T. S.; Haupt, K. *Adv. Mater.* **2013**, *25*(7), 1048.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Analysis Of Synergistic Anticancer Effects of Sorafenib and *Cynara scolymus* Extract on HEPG2 Liver Cancer

Uğur DURMUŞ, Nihan GÜNAY, Mehmet KUZUCU

Department of Biology, Faculty of Sciences and Art, Erzincan Binali Yıldırım University

ugurmoleculer@gmail.com

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death in the world, affecting more than 500,000 people. HCC is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis[1].

Sorafenib belongs to a group of drugs called protein kinase inhibitors used in the treatment of Hepatocellular carcinoma. This is the first FDA-approved systemic therapy for patients with advanced HCC not amenable to treatment by surgical resection or liver transplantation. Sorafenib is a biaryl urea that blocks the Raf/MEK/ERK pathway by inhibiting Raf serine/threonine kinase isoforms[2].

Cynara scolymus (artichokes), widely consumed as part of a traditional Mediterranean diet, yield one such macronutrient, artichoke leaf extract (ALE). Studies have shown that has shown potential as a lipid lowering and hepatoprotective agent. However, the effect of ALE, which is frequently used by liver patients, in the treatment of liver cancer is not yet known[3].

In this study, we examined the synergistic effect of Sorafenib and ALE on liver cancer HEPG2 cell. Sorafenib and ALE will be administered separately and in combination to HEPG2 cells and the combination index (CI) values of the results were calculated. Expression levels of some apoptosis genes (Caspase-9, Bax and Bcl-2) will be determined by RT-qPCR at the highest CI. Protein analysis of Caspase-9, Bax and BCL-2 were performed by ELISA. We determined the levels of total antioxidant capacity and the activity of the antioxidant enzymes glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase in the Sorafenib and ALE-applied cells.

References

Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin. 2005;55:74–108.
Xie, B., Wang, D. H., & Spechler, S. J. (2012). Sorafenib for treatment of hepatocellular carcinoma: a systematic review.Digestive diseases and sciences, 57(5), 1122–1129. doi:10.1007/s10620-012-2136-1.

Rondanelli, M., Giacosa, A., Morazzoni, P., Guido, D., Grassi, M., Morandi, G., … Perna, S. (2016). MediterrAsian diet products that could raise HDL-cholesterol: A systematic review. BioMed Research International, 2016, 2025687.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Inhibition Effect of Some Benzoate Derivatives on Bovine Lactoperoxidase Enzyme

Işıl Nihan ÖZYÜREK [a], Nurgül ABUL[a], Serpil GERNİ[a], Ramazan KALIN[b], Ali ATASEVER[c], İlhami GÜLÇİN[a], Hasan ÖZDEMİR[a]

[a]*Atatürk University, Faculty of Science, Department of Chemistry, 25240-Erzurum, Turkey*
[b]*Department of Basic Science, Faculty of Sciences, Erzurum Technical University, 25240-Erzurum, Turkey*
[c]*Ataturk University, Ispir Hamza Polat Vocational Training School, 25240-Erzurum, Turkey*

ramazan.kalin@erzurum.edu.tr

Peroxidase enzyme isolated from milk is called as lactoperoxidase (LPO, E.C. 1.11.1.7). It is the first enzyme reported in milk with oxidoreductase activity. [1-2] LPO is found in saliva, tears, biological fluids and commonly found in milk. Its main task is to catalyze the oxidation of molecules in the presence of hydrogen peroxide to produce products with broad antimicrobial activity. Pseudo halogen, thiocyanate or halogens are required to act as the second substrate for the enzyme to exhibit this antimicrobial effect.[2-3]

The aim of this work is to investigate the in vitro effects of some methyl 2-amino benzoate derivatives on LPO activity. For this purpose, LPO was first purified in a single step with high yield by using Sepharose-4B-L-Tyrosine-sulfacetamide affinity chromatography. In order to determine the inhibition effects of the benzoates, spectrophotometric measurements were performed at 412 nm in the presence of ABTS chromogenic substrate. Activity (%)-[Benzoate derivatives] graphs were drawn and IC50 values were calculated from this graph. Inhibition type and Ki values were determined from the equations obtained from the Lineweaver-Burk graphs.[4]

The IC50 values were calculated as 0.044, 8.663 and 17.769 µM for methyl 2-amino 3-bromo benzoate, methyl 2-amino 4-bromo benzoate and methyl 2-amino 4-chloro benzoate, respectively. On the other hand, Ki values from Lineweaver-Burk graphs were calculated as 0.096 ± 0.02, 5.249 ± 0.48 and 40.339 ± 8.23 µM, respectively. It was found that while methyl 2-amino 3-bromo benzoate and methyl 2-amino 4-bromo benzoate exhibited non-competitive inhibition, methyl 2-amino 4-chloro benzoate showed competitive inhibition.

References:

Sisecioglu, M.; Cankaya, M.; Gulcin, İ.; Ozdemir, H. *J. Enzyme Inhib. Med. Chem.* **2010,** 25(6), 779.
Atasever, A.; Ozdemir, H.; Gulcin, İ.; Kufrevioglu, Ö. İ. *Food Chem.* **2013,** 136(2), 864.
Reiter, B.; Perraudin, J. P. Lactoperoxidase: biological functions. In Peroxidases in Chemistry and Biology, 1. CRC Press, Boca Raton, FL, 143, Canada, USA, 1991.
Koksal, Z.; Kalın, R.; Gulcin, İ.; Ozdemir, H.; Atasever, A. *Int. J. Food Propert.* **2016,** 19, 1207.

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Levan Production Potential of The Halophilic Isolates

Hakan Çakmak[a], Seval Çınar[b], Emir Zafer Hoşgün[c], Mehmet Burçin Mutlu[b], Pınar Aytar Çelik[a]
Ahmet Çabuk[a]

[a]*Eskisehir Osmangazi University, Department of Biotechnology and Biosafety, Eskisehir, Turkey*
[b]*Eskisehir Technical University, Department of Biology, Eskisehir, Turkey*
[c]*Eskisehir Technical University, Deparment of Chemical Engineering, Eskisehir, Turkey*
ahmetcabuk@gmail.com

Exopolysaccharides (EPS) are an increased attraction because of industrial and medical applications Among of microbial polysaccharides, levan is a β(2-6)-linked fructose homopolysaccharide that is extracellularly produced from sugar-based substrates by a variety of microorganisms.

In this study, the halophilic strains examined in this study were previously obtained from different hypersaline environments in Turkey: solar salterns in Tuzlagözü (Sivas), Fadlum (Sivas), Kemah (Erzincan), a hypersaline spring water in Pülümür (Tunceli) and a saline lake in Delice (Kırıkkale). 12 isolates were screened for levan production on the basal medium. After incubation and ethyl alcohol treatment, dialysis process was operated for partial purification. Levan amounts in our samples were calculated based on the amount of sugar obtained by acid hydrolysis of standard levan. The levan samples and the standard were subjected to acid hydrolysis. Then, the pH of the mixture was neutralized. Sugar amount of samples were determined using HPLC system. ^{1}H-NMR spectra of the levan sample and standard were recorded.

In our study, the results obtained by high performance liquid chromatography (HPLC) analysis showed that *Chromohalobacter canadensis* strain 85B had highest production potential as 234.67 mg levan/g biomass. The chemical shifts of proton NMR spectra of the isolated levan also exhibited high similarity to those of levan isolated from *Erwinia herbicola.*

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Exosome Isolation from Subventricular Zone

Can Berk CAMCI[1*], Sıla BİLGİN[1], Burak DERKUŞ[2], Emel Emregül[1]

a Ankara University, Faculty of Science, Department of Chemistry and PMBR Lab. Ankara TURKEY
[1] *Ankara University, Faculty of Science, Department of Chemistry, PMBR Lab. Ankara Turkey*

[2]Eskişehir Osmangazi Üniversitesi Mühendislik Mimarlık Fakültesi Biyomedikal Mühendisliği Bölüm

[*]*Corresponding author: Can Berk CAMCI, canberkcamcii@icloud.com*

The subventricular zone and dentate gyrus are the most dense areas for neural progenitor cells. This study aimed at using the waste media for the isolation of exosomes obtained from cultures of neural progenitor cells following the isolation of the two aforesaid regional tissues.

In this study, the waste medium was sequentially centrifuged at 110000g and 4ºC by means of the ultra-centrifugation method followed by separation of the precipitate and supernatant phases. The aim of sequential centrifugation was to remove and seperate various unwanted biochemical products (protein, RNA, lipid) from the waste media. The success of the isolation method was investigated using TEM, WesternBlot and Bradford tests for exosomes resuspended in PBS buffer.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effects of Pineapple Derived Exosomes on Wound Helaing

İrem Özkan[a], Oğuz Kaan Kırbaş[a], P. Neslihan Taşlı[a], Fikrettin Şahin[*]

[a] Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, 26 Ağustos Campus, Kayisdagi cad., Kayisdagi, TR-34755 Istanbul, Turkey.

*Coresponding Author. Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Yeditepe University, 26 Ağustos Campus, Kayisdagi cad., Kayisdagi, TR-34755 Istanbul, Turkey.

E-mail: (fsahin@yeditepe.edu.tr)

Wound healing, as a main physiological process, has been extensively studied for decades but a non invasive treatment methods are yet to be routinely used in clinic due to high costs or patient specific response of the developed treatments. Secreted from cells to communicate, exosomes are nanosized vesicles with their sizes ranging between 30-100 nm. Although it is has been widely studied that eukaryotic cells communicate through exosomal pathways, the role of plants exosomes remain unexplored [1,2]. Here, we examined for the first time the effects of pineapple exosomes on wound healing *in vitro*. Therefore, we first characterized the exosome-like nanovesicles derived from pineapple juice via the exosomal markers with flow cytometry, size and distribution with Nanoparticle tracking analysis (NTA) and exosomal uptake of the cell. In order to evaluate the effects of these exosomal nanovesicles on wound healing we performed scratch assay on human neonatal foreskin cells (ATCC PCS-201-010) and tube formation via HUVECs [3]. We also evaluated gene expression levels of these cells for cell proliferation and wound healing capabilities of pineapple derived exosomes.

Keywords: Exosomes, Wound Healing, Plant derived nanovesicles

References:

[1] J. Mu et al., “Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles,” Mol. Nutr. Food Res., vol. 58, no. 7, pp. 1561–1573, 2014.

[2] S. Ju et al., “Grape Exosome-like Nanoparticles Induce Intestinal Stem Cells and Protect Mice From DSS-Induced Colitis,” Mol. Ther., vol. 21, no. 7, pp. 1345–1357, 2013.

[3] Ilic D., Polak JM., 2011. Stem cells in regenerative medicine: introduction. British Medical Bulletin. 98117–126.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Preparation of the Electrospun Poliurethane/Polycaprolactone Fiber Modified Screen-Printed Carbon Electrodes for Electrochemical Determination of Serotonin

Ensar EREL, Ayşe Başak ÇAKMEN, Süleyman KÖYTEPE, Burhan ATEŞ, İsmet YILMAZ
İnönü University, Department of Chemistry, Malatya, TURKEY
email: ensarerel@gmail.com

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter and regulates mood, sleep, emesis (vomiting), sexuality and appetite [1]. In the absence of serotonin, depression, fatigue, stress, anxiety and migraine may occur [1]. Structurally, it belongs to the monoamine group and is synthesized from the amino acid tryptophan. Rapid and accurate detection of serotonin is important in the detection and monitoring of many diseases. Thus, in this study, poliurethane/polycaprolactone (PU/PCL) based electrodes were developed to detect serotonin. Firstly, polyurethane film was synthesized from hexamethylene diisocyanate, trimethylolpropane ethoxylate (TMPE450), polyethylene glycol 200 and Tween 40 and characterized with different instrumental techniques. Then, nanofibers were prepared by electrospinning process from prepared PU and polycaprolactone (PCL) mixture. This nanofibers were formed by coating of PU/PCL (1/3 ratio) mixture on screen-printed carbon electrode to prepared serotonin selective electrode. Structural characterizations of PU/PCL nanofiber films were characterized by FTIR, DTA, TGA, SEM and dynamic contact angle measures. Serotonin sensing properties of the PU/PCL modified electrode were performed with cyclic voltammetric (CV) and differential pulse voltammetric measurements. The electrospun fibre morphology and electrochemical sensitivity were investigated and optimized. The voltammetric results indicate that the polyurethane based electrodes can be used as a sensor for determination of serotonin with the good sensitivity, selectivity, high reproducibility and high R-value.

We would like to thank Inonu University BAP for financial support within the project numbered İ.Ü.BAPB-FYL-2018/1073.

[1] I. Cesarino, H.V. Galesco, S.A.S. Machado, Materials Science and Engineering: C, 40, 2014, 49-54,

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effect of Quercetin in BRAF Gene Suppressed Prostate Cancer Cells

Şevval DEMETGÜL, Hatice ÇELİK, Havva Nur ÇANAK, Serdar KARAKURT

Selçuk University Faculty of Science, Department of Biochemistry Konya / Turkey

sevvaldemetgul3@gmail.com

Cancer is one of the most important health problems encountered in the last century, especially in terms of high mortality and incidence. Conventional methods used to treat Prostate Cancer (PCa), the most common type of cancer in men, both cause serious side effects and have inadequate effects. Therefore new and alternative treatment methods need to be developed. Recently, the efficacy of phenolic and flavonoid compounds in plant and plant extracts against various types of cancer has been investigated and one of the most studied among these flavonoids is quercetin. However, both the problems of stability and the detoxification mechanisms of the cells and the quercetin do not show sufficient effect. In this study, the suppression of the oncogenic BRAF gene, which plays an important and active role in the development of prostate cancer, and the changes that may occur in cytotoxic effect on prostate cancer of the combined effects of quercetin were examined. Prostate cancer cell line; After the gene was suppressed with BRAF gene-specific siRNA in PC-3 cells, BRAF(-) cells were transferred to 96-well-stocked plates and treated with quercetin in the range of 0 to 200°M and incubated at 48 hours 37°C. Cell vitality and cytotoxic effect scanned with Alamar blue. Prostate cancer cell PC-3 's treatment with quercetin result was also observed a dose-dependent inhibition. IC50 was calculated as 161°µM. In BRAF gene-suppressed PC-3 cells, the cytotoxic effect of quercetin in cytotoxicity increased by 18% and the IC50 decreased to 133. Quercetin, one of the natural compounds of the plant, has a cytotoxic effect against many cancer cells. Quercetin cytometry, which has been increased by suppression of the BRAF gene, which is oncogenic, maybe a new approach to prostate cancer treatment as a result of protein-xenobiotic interaction. This study was supported by TUBITAK (Project number: 1919B011802766)

KEYWORDS: Prostate cancer, BRAF gene, Quercetin, Combined therapy

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Effect of Adenosine Triphosphate on Methanol Induced Oxidative Liver Injury in Rats

Yunus KOÇ[a], Cuma Mertoğlu[a] , Halis Süleyman[b], Murat Çankaya[c], T. Abdulkadir Çoban[a]

[a]Department of Biochemistry, Faculty of Medicine, Erzincan Binali Yıldırım University

[b]Department of pharmacology, Faculty of Medicine, Erzincan Binali Yıldırım University

[c]Department of Biology, Faculty of Art and Science, Erzincan Binali Yıldırım University

acoban@erzincan.edu.tr

Methanol (methyl alcohol) is a colorless, volatile and a type of alcohol whose metabolites are toxic. In our country, it has been found that methyl alcohol intoxication has been observed to a certain level over the years. Methyl alcohol has serious clinical consequences, from blindness to death. The primary toxic factor in methyl alcohol intoxication is metabolic acidosis. Many studies have investigated the effect of methyl alcohol on hepatotoxicity due to oxidative damage. Although adenosine triphosphate (ATP) has been shown to be effective in studies on ischemic tissue to reduce oxidative damage, there is no study on its effect on oxidative damage in methyl alcohol-induced hepatotoxicity. In this context, we aimed to investigate the protective effects of ATP on alcoholic acute liver injury explained by oxidative stress in rats.

A total of 24 rats were randomly divided into 3 groups. Group 1 was selected as the control group and no procedure was performed. After oral administration of methotrexate 0.3 mg / kg by gavage to groups 2 and 3 for 7 days, 20% methanol was given in the same route at 3 g / kg. Blood was taken from the tail veins of the animals for the measurement of serum ALT and AST 8 hours after ATP injection. Subsequently, the animals were sacrificed by high dose (50 mg / kg) thiopental anesthesia and histopathological examinations were performed on the excised liver tissues.

ALT, AST values and AST / ALT ratio were found to be higher in the methanol-treated group than the other two groups and higher in the methanol + ATP group than the control group ($p < 0.001$). When the tissues were evaluated histopathologically; normal polygonal cells with prominent round nuclei and eosinophilic cytoplasm were observed in the liver control tissue. Kupffer cells were distributed between hepatocyte cell cords and the vessels were in normal structure. The most significant change in the MTO group was hepatocyte degeneration and hepatic cord separation. Hepatocytes were swollen and showed severe hypertrophy, Kupffer cell number was relatively increased and hepatocyte cytoplasms contained vacuoles. Polymorphonuclear cell infiltration was also observed around the central vein. Hepatic cords were normal in MAP group and degeneration in hepatocytes was low. Kupffer cells were relatively reduced in number and a small amount of polymorphonuclear cells were observed in the central veins. As a result, methanol hepatotoxicity can be reduced with ATP treatment.

This investigation suported by Erzincan Binali Yıldırım University Scientific Research Fund (TYL-2019-618)

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

The Preparation of Antibacterial Wound Dressing Materials Containing Allantoin via Electrospinning Method

Ayşe Başak ÇAKMEN[1], Samir Abbas Ali NOMA[1], Canbolat GÜRSES[2], Süleyman KÖYTEPE[1], Burhan ATEŞ[1], İsmet YILMAZ[1]

[1]*Inonu University, Faculty of Arts & Sciences, Department of Chemistry, Malatya, TURKEY*
[2]*Inonu University, Faculty of Arts & Sciences, Molecular Biology and Genetics, Malatya, TURKEY*

E-mail: basak_cakmen@hotmail.com

Wound dressing materials are used extensively today and they are a field of study in which our country is dependent on other countries. Although there are various wound dressing materials, there are still some studies on wound dressing materials on which have more optimum conditions in wound healing. In the preparation of wound dressing materials, materials developed via electrospinning method have come to the forefront day by day. The aim of this study was to prepare wound dressing materials via electrospinning technique using polyurethane, polycaprolactone and crosslinked PEGs, and also to obtain a multifunctional structure with the addition of allantoin known as a wound healer and gentamicin sulfate known as having antibacterial effect into the wound dressing material.

In the scope of this study, three polyurethanes with different tween ratios were synthesized using hexamethylene diisocyanate (HDI), polyethylene glycol (PEG 200), trimethylolpropane ethoxylate (TMPE 450) under an inert atmosphere at 70°C in a reflux system. 3% allantoin and 1% gentamicin sulfate were added into the mixture prepared with a polyurethane/polycaprolactone ratio of 1:3. The obtained structures were characterized structurally by FTIR, morphologically by SEM and AFM, thermally by TGA, DTA and DSC. The characterized polymers were transformed into a wound dressing material via electrospinning under optimum conditions using the flow velocity parameters as 20 kV and 2 mL/h with 20 cm away. The wound dressing materials were found to be biocompatible against L-929 cells in the cell culture system and allow the cells to adhere.

The biomedical usable nature of the wound dressing material obtained from the study is important in terms of creating a domestic product and providing an accretion value for our country.

This study was supported by İnönü University BAP unit with İÜ.BAPB-FYL-2018/1073 project.

References

Unnithan, A. R.; Sasikala, A. R. K.; Murugesan, P.; Gurusamy, M.; Wu, D.; Park, C. H.; Kim, C. S. Electrospun polyurethane-dextran nanofiber mats loaded with Estradiol for post-menopausal wound dressing. Int. J. Biol. Macromol. 2015, 77, 1–8. DOI: 10.1016/j.ijbiomac.2015.02.044.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

GEMCITABINE LOADED & MAGNETICALLY RESPONSIVE CHITOSAN AND TRIMETHYL CHITOSAN NANOPARTICLE DEVELOPMENT AND IN VITRO EVALUATION FOR LUNG CANCER CELL LINES

Ayşe Ünal[a], Güliz Ak[a,b], Şenay Hamarat Şanlıer[a,b]

[a]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[b]*Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

ayseunal.93.35@gmail.com

Chitosan is native polysaccharide and can be obtained by partial deacetylation of chitin. Chitosan is biodegradable and non-toxic polymer. However chitosan has poor aqueous solubility. For these reason, chitosan derivatives is used. Trimethyl chitosan (TMC) is one of the methylated derivative of chitosan. Gemcitabine is hydrophilic cancer drug of the anti-metabolite class. It interferes with DNA synthesis by including elongating DNA and indirectly interferes with DNA replication. It is used for various carcinomas such as lung carcinoma, etc. but after the administration, some patients may experience serious complications. Nanoparticle-based therapeutic agents have been developed for use in cancer therapy. Magnetic nanoparticles are moved magnetic field and distracted easily from the medium. In this study, we tried to develop two different drug delivery systems that could direct drugs to target cancerous tissue so magnetic nanoparticles was used.

Gemcitabine was linked to chitosan and TMC nanoparticle and these nanoparticle drug conjugates were attached to magnetite. Magnetite nanoparticles were synthesized using co-precipitation method and magnetic TMC nanoparticles (MTMCs) and chitosan nanoparticles were prepared by cross-linking method and gemcitabine was loaded via adsorption technique. After characterization, in vitro drug release and cytotoxicity studies were carried out. FTIR result showed that trimethyl chitosan had C-H peak in 2850 cm^{-1} but chitosan has not. After that appropriate amounts of TPP, magnetite and gemcitabine were determined. Adsorption of 1.5 mg/mL gemcitabine onto MTMCs was carried out with yield of 54%. Moreover chitosan was absorb 2.5 mg/mL gemcitabine with yield of 30.3%. Both carrier system had controlled drug release profile. According to all these data, trimethyl chitosan nanoparticles have a potential for further analysis (in vivo, ex vivo) as a magnetic targeted therapy agent for lung cancer treatment.

Acknowledgement: We would like to thanks for financial support received from Ege University (Project ID: 2013 FEN 031).

Keywords: Chitosan, Trimethyl Chitosan, Gemcitabine, Magnetic Nanoparticle, Drug Delivery System, Drug Release, Lung cancer

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Methotrexate Loaded Chitosan Nanoparticles In Psoriasis

İlay TOZAK[a], Güliz AK[a,b], Şenay Hamarat ŞANLIER[a,b]

[a]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*

[b]*Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmır, Turkey*

tozakilay@gmail.com

Psoriasis is an immune-mediated, genetic disease manifesting in the skin or joints or both. It is a chronic inflammatory skin disease characterized by a significant deterioration in the quality of life of affected individuals. In patients with psoriasis has need appropriate effective treatment and long-term disease control[1]. Methotrexate typically is only used to treat severe cases of psoriasis, when the symptoms are debilitating. Methotrexate inhibited the enzymes dihydrofolate reductase(DHFR) and thymidylate synthase, as well as other enzymes involved in de novo purine synthesis. Methotrexate deplete the intracellular reserve of fully reduced folates, and thus affect transmethylation reactions[2]. Chitosan nanoparticles have gained more importance as drug delivery carriers because of their better stability, low toxicity, simple preparation method and providing versatile routes of administration[3]. In this study it is aimed to develop a transdermal usable form of methotrexate for psoriasis patients. For this prupose, chitosan nanoparticles were synthesized and methotrexate loaded. After that, the gel formulation was developed and characterized. Finally, the usage potential of the obtained gel formulation was investigated.

References:

Türkiye Psoriasis Tedavi Kılavuzu-2016, www.turkderm.org.tr (Erişim Tarihi: 13.09.2019)
Dogra, S.; Mahajan, R, 2013, Systemic methotrexate therapy for psoriasis: past, present and future. Clinical and Experimental Dermatology, 38(6), 573–588.

Tiyaboonchai, W., 2003, Chitosan nanoparticles : A promising system for drug delivery, Naresuan University Journal, 11(3): 51-66.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Glioblastoma Tumor Model In Nude Mice

Özge Sarı, Ayşe Ünal,[1] Tuğba Karakayalı,[1] Güliz Ak,[1,2] Şenay Hamarat Şanlıer[1,2]

[1]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[2] *Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

sariozge86@gmail.com

Glioblastoma is the most malignant tumor of the central nervous system in adults and accounts for 50-60% of all gliomas and 22.6-27% of primary brain tumors. The tumor is histologically and genetically heterogeneous. Glioblastoma is a stage 4 tumor according to World Health Organization classification, characterized by the presence of necrosis and microvascular proliferation as well as malignant pleomorphic astrocytic cells exhibiting advanced nuclear atypia and mitotic activity. It mostly affects people over 50 years of age. Radical surgical resection is an appropriate treatment approach as an initial treatment option and adjuvant treatments such as radiotherapy and chemotherapy may be added. Despite many treatment approaches, the prognosis of patients with glioblastoma is poor and the average survival is approximately one year. For this reason new treatment strategy must be developed. In this work, in vivo glioblastoma tumor model was established to provide the basis for future studies. For this purpose, glioblastoma cells with U87-MG-Red-Fluc luciferase activity was used for brain tumor formation in CD1 nude male mice. Then, mice would be placed in the stereotactic frame under ketamine and xylazine anesthesia. Once the scalp is opened, cell suspension was injected to the right striatum using a Hamilton glass syringe. The needle was move very slowly as the tip of the needle was retracted. Mice would be monitored from day one of U87 cell implantation to monitor their daily activities and general condition. After evaluations, the mice would be sacrificed from the surrounding area, which cannot be fed on their own, blushing their eyes and do not respond to external stimuli (Geletneky K., 2010).

Acknowledgements

We would like to thanks for financial support received from TUBIAK (Project ID: 117 S 241).

Keywords: Glioblastoma, tumor modelling, stereotactic frame, nude mice, U87-MG-Red-Fluc.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Pemetrexed Loaded Alginate-Chitosan Nanoparticles And Investigation Drug Release Profile

Ayça EREK[1], Safiye Kübra AKTAŞ[1], Yağmur ÖZDEMİR[1], Güliz AK[1,2], Şenay Hamarat Şanlıer[1,2]

[1]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[2] *Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

erekayca@gmail.com, skubraaktas@gmail.com

Lung cancer is a type of cancer that begins in the trachea, bronchus or lung tissue. It is the most common cause of cancer death. Lung cancer can occur for several reasons. Although chemotherapy is the most widely used method in lung cancer, it has drug resistance and many life - threatening side effects. For this reason, there is a need to develop new more specific treatment methods. The aim of this study is to minimize or reduce the side effects of treatment[2]. In this work chitosan and alginate were preferred because of their biodegradability, biocompatibility and non-toxic properties[3-4]. Pemetrexed is selected as a chemotherapy agent since it is a highly targeted anti-cancer antifolate that acts by interrupting important folate-dependent metabolic processes required for cell proliferation. [5] In this study, alginate nanoparticles were synthesized by induced gelation method and their surfaces were coated with chitosan. Then pemetrexed was loaded at different concentrations into these alginate/chitosan nanoparticles by adsorption method and optimum concentration was determined. Furthermore, final formulation of nanoparticles were characterized with FTIR (Fouirer Transform Infrared Spektrofotometre) and SEM (Scanning Electron Microscope). The size of the nanoparticles was about 80 nm. Drug loading yield was calculated as 57.80 % for optimum concentration as 2 mg/ ml. Finally, drug release studies were performed at both pH 7.4 and pH 5.5. This study was shown that Pemetrexed loaded alginate-chitosan nanoparticles have a potential for advancing in cancer treatment studies.

References:

1) Cancer Research UK," Lung Cancer, Treatment", https://www.cancerresearchuk.org/about-cancer/lung-cancer/treatment (Erişim Tarihi: 25 Şubat 2019)

2) İşoğlu S.D., Kanserde İlaç Hedefleme, Biomedya, 1(4):14-15s.

3) Paques, J.P., Linden, E., Rijn, C.J.M. and Sagis, L.M.C., 2014, Preparation methods of alginate nanoparticles, Advances in Colloid and Interface Science, 209:163-171pp.

4) Lizardi-Mendoza, J., Monal, W.M.A. and Valencia, F.M.G., 2016, Chemical characteristics and functional properties of chitosan, Chitosan in the Preservation of Agricultural Commodities, 1:3-31pp.

5) Rollins, D.K. and Lindley, C., 2005, Pemetrexed: A Multitargeted Antifolate, University of North Carolina School of Pharmacy, Clinical Therapeutics 27(9):1343-1375pp.

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

In Vivo Biodistribution Of Temozolomide and Carmustine Loaded & Targeted Solid Lipid Nanoparticle

Şenay Hamarat Şanlıer,[a,b] Ayşe Ünal,[a] Tuğba Karakayalı,[a] Güliz Ak,[a,b] Özge Sarı,[1]

[a]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[b] *Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

senay.sanlier@gmail.com

In 2014, one of the causes of cancer deaths before the age of 40 was brain cancer. According to the American Brain Tumor Association, the most common cancer type after leukemia in individuals aged 0 to 19 years is brain tumor. Glioblastoma multiform, which is the most common primary brain tumor, constitutes 40% of malignant brain tumors in addition to limited treatment options. Glioblastoma cells are predominantly composed of abnormal astrocytic cells, but also include different cell types and areas of necrotic cells, so treatment is very difficult. The first step in the treatment of glioblastoma is the surgical procedure to remove the tumor. The next step is radiotherapy and chemotherapy. Carmustine, which is in the class of alkylating agent, causes apoptosis by causing DNA damage. There are many side effects such as pulmonary toxicity, nausea, vomiting, dizziness and loss of coordination. Temozolomide is an alkylating agent and works by stopping cancer cells from making DNA. These drugs used in treatment can increase life expectancy by 2.5 months on average. For this reason new treatment approaches need to be developed. In this study, MCT1 targeted solid lipid nanoparticles containing dual drugs for glioblastoma treatment were developed and biodistribution was examined in vivo**.** It is aimed to determine in which organ the drugs which are the active substances of the system are deposited. Therefore, mice were sacrificed at the first and third hours (n = 3) after intranasal administration. Then heart, liver, spleen, kidney, brain and blood samples were collected. The organs were homogenized with methanol and PBS. After homogenization, TMZ and BCNU analysis was performed by HPLC method in all organ and blood samples. According to HPLC result, spleen, heart, lung samples could not be detected drug. It can be said that there is no accumulation of solid lipid nanoparticles containing drugs in these organs. However, when the drug analysis is performed in brain and kidney, accumulation is seen.

Keywords: Solid lipid nanoparticle, Temozolomide, Carmustine, Glioblastoma, Intranasal application, Blood-brain barrier.

Acknowledgements

We would like to thanks for financial support received from TUBIAK (Project ID: 117 S 241).

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Remeoval Of Dye With Using Chitosan Nanoparticles

Zeynep Gaye Hamarat[a], Güliz Ak[b,c], Şenay Hamarat Şanlıer[b,c]

[a]*Biology Department, Faculty of Science, Ege University, Izmir, Turkey*
[b]*Biochemistry Department, Faculty of Science, Ege University, Izmir, Turkey*
[c]*Center for Drug Research & Development and Pharmacokinetic Applications, Ege University, Izmir, Turkey*

gayehamarat@gmail.com

In this century, environmental pollution is one of the most serious problems facing humanity and other life forms on our planet. Environmental pollution affects biodiversity, ecosystem and human health worldwide by contaminating waters. One of the specific contaminants presents in these days is synthetic dyes. There are used in paint, textile, paper and food industry. These dyesresult with colored waste waterand they may cause carcinogenic and genotoxic compound formation by causing chemical reactions. In order to prevent negative impacts on the environment, dyes have to be removed from waste water. For this aim, coagulation, precipitation, membrane filtration, adsorption methods are widely used and many materials are being investigated as an alternative.ın our study, chitosan nanoparticles were synthesized using ionic gelation methods in order to use removal of sunset yellow dyes from aqueous solution in a batch system. It was concluded that the 52.4% adsorption with 24 hours. It showed that the chitosan nanoparticles had good performance for removal of sunset yellow and could be used as a highly efficient adsorbent to treat dyes containing waste water.

Key Word: Chitosan, chitosan nanoparticle, dye, sunset yellow, waste water treatment

2. EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

From Glass to Drug: Silica Nanoparticles Against Prostate Cancer Proliferation

İrem Mukaddes DURMUŞ[a], İlyas DEVECİ[b], Serdar KARAKURT[a]

[a]*Selcuk University, Faculty of Science, Department of Biochemistry, Konya, TURKEY*

[b]*Konya Technical University, Vocational School Of Technical Sciences, Chemistry and Chemical Processing Technologies, Konya, Turkey*

iremdurmuss73@gmail.com

Cancer is a deadly disease and its treatment is often limited and low because of the lack of targeted treatment options in the success of conventional treatments such as chemotherapy and radiation therapy. Therefore, targeted management of anticancer agents is important to overcome limitations in treatment. Advances in nanoparticle (NP) based drug delivery systems have been shown to disperse drugs in the cytoplasm by cell membrane targeting. The synthesized silica-amine nanoparticle complex (SiNP-NH_2) was used for this purpose to investigate its toxic effect on prostate cancer cell line (PC-3) and prostate healthy epithelial cell (PNT1A). The PC-3 cell line was grown in DMEM High Glucose and the PNT1A cell line was grown in RPMI 1640 growth medium. Cells that reached sufficient density were seeded in a 96-well plate with 10^4 cells per well. After incubation for 24 hours at 37 °C, 95% humidity and 5% CO_2, the cells were treated with SiNP-NH_2 complex at a dosage range of 0-250 µm. After 48 hours of incubation, cell viability was determined by Alamar Blue and the IC_{50} value was calculated. For cell imaging, the PC-3 cells were seeded in 4-well plates and fixed by fixation for half an hour of SiNP-NH_2 treatment followed by imaging on the BioRad, ZOE fluorescence imaging device. In addition, in order to determine in which way SiNP-NH_2 leads PC-3 cells to death, controlled cell death mechanism; apoptosis studies were performed with NovoCyte Flow cytometer system using Annexin V-APC and 7-AAD dyes. IC_{50} value was examined for SiNP-NH_2 was calculated as 153.4 µM. On the other hand, no toxic effect was found against PNT1A cells. The synthesized SiNP-NH_2 6-fold increased the early apoptosis. In summary, exposure to SiNP-NH_2 results in a dose-dependent cytotoxicity in human prostate cancer cells that is closely correlated to increased apoptosis.

Keywords: Silica Nanoparticles, Prostate Cancer, Cytotoxicity, Cell Imaging, Apoptosis

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Upregulation of Caspase Gene Expression in Human Prostate Cancer by Tannic Acid

Havva Nur ÇANAK, Serdar KARAKURT

Selçuk University,Science Faculty, Department of Biochemistry, Selcuklu/KONYA

hnur.canak@gmail.com

Tannic Acid (TA) is a naturally occurring herbal polyphenolic compound with antioxidant, antimutagenic, anticarcinogenic, antimicrobial, anticarcinogenic effects. Prostate cancer (PCa) is one of the most important causes of death in the world, especially in men, and is 4th in morbidity and mortality. Prostate cancer cells rearrange gene and protein expressions at the molecular level, allowing cells to multiply uncontrollably. Synthesis of caspase enzymes that have an active role in the pathway of apoptosis, one of the mechanisms of cell death, has been modified in prostate cancer cells. The aim of this study was to investigate the effects of TA on the expression of caspase genes on PC-3 from human prostate cancer cells. For this purpose, PC-3 cells were grown in DMEM growth medium at 37 ° C under 5% CO_2 conditions and the cells were then treated with 0-200 µM TA and the IC_{50} value was calculated from the sigmoidal graph using Alamar Blue reactant. The mRNA expressions of caspase 1, caspase 3, caspase 4, caspase 5, caspase 6, caspase 8, caspase 10 genes from the total RNA obtained from PC-3 cells treated with IC_{50} value were determined by qRT-PCR method. TA suppressed the proliferation of PC-3 cells in a dose-dependent manner and IC_{50} value was found the 35.3 µM. MRNA expression of caspase genes as a result of TA treatment, while Caspase 1,3,5,6 and 8 mRNA expressions were suppressed, caspase 4 and caspase 10 increased. In this study, it has been shown that increased apoptosis after TA treatment occurs as a result of with TA modification of Caspase genes. In particular, TA may play an important role in the regulation of caspase genes against proliferation of cancer cells and offer a new therapeutic approach.

Keywords: Tannic Acid, Prostate Cancer, Apoptosis Pathway, Gene Expression, Micro Array

2.EBAT EURASIA BIOCHEMICAL APPROACHES & TECHNOLOGIES CONGRESS
26-29 OCTOBER 2019 ANTALYA

Production of pullulanase from *Fontibacillus pullulanilyticus* DSHK107^T

Dimla Sihay, İlker Kaya, Mete Karaboyun, Hatice Korkmaz Güvenmez
Cukurova University, Science and Letters Faculty, Biology Department, Adana, Turkey
Email address: hkorkmaz@cu.edu.tr

Pullulan is an extracellular and neutral polysaccharide produced from starch by strains of Aureobasidium pullulans.[1] Pullulanases (EC3.2.1.41) catalyze mainly the hydrolysis of (1→6)-α-D-glycosidic linkages in pullulan.[2] In this study, pullulanase was produced by a new type of genus of *Fontibacillus*; *Fontibacillus pullulanilyticus* DSHK107^T isolated from soil of Cukurova University campus and the produced enzyme has been characterized. Total 110 strains were inoculated into producing medias (%1 starch and %1 pullulan contained) and incubated at 37°C for 72 hours. Then for the detection of pullulanase activity the bacteria culture was stained with iodine solution. 14 of them showed pullulanase activity. The strain showing highest pullulanase activity was used to produce pullulanase. After some partial purificiation steps, a crude enzyme extract of *F. pullulanilyticus* DSHK107^T containing pullulanase activity was obtained.

The partially purified pullulanase has optimum activity at pH 6.0 and 40 °C. The pullulanase was highly stable at pH values between 4.0-7.0 and temperature between 30-50 °C. According to these results due to the characteristics of the enzyme, it can be suggested that industrial field use is appropriate.

Acknowledgements: This study was supported by Scientific Research Projects Unit of Cukurova University with the project number of FYL-2014-2634.

References:

Leathers, T.D. App. Microbiol. Biotechnol. **2003**, 468.
Kuroiwa, T.; Shoda, H.; Ichikawa, S.; Sato, S.; Mukataka, S. Process Biochem. **2005**, 2637.


nehir
BİYOTEKNOLOJİ


KEÇİÖREN
BELEDİYESİ
KEÇİÖREN
BELEDİYESİ


redoks
LAB


Leica
MICROSYSTEMS


TETRA
TEKNOLOJİK SİSTEMLER A.Ş.
www.tetratek.com.tr


ZEISS


TERRA


EpiGen
DNA


TARMAY KİMYA


LabMedya


PALME
YAYINEVİ


ZK
ZEKİ KIRTASİYE A.Ş.


Merkez
Çadır Branda


CHEMLIFE


labkon
Laboratuvar Ekipmanları


ALFAGEN


KURUKAHVECİ
KURUKAHVECİ
MEHMET EFENDİ


ChromaScience
Kimya Teknolojileri


sare


ADEN BİYOTEKNOLOJİ
LABORATUVAR SİSTEMLERİ LTD. ŞTİ.


MST
MedSanTek
LABORATUAR MALZEMELERİ SAN. VE TİC. LTD. ŞTİ.