Sarajevo ICABB 2021 CONGRESS



5th International Congress on Advances in Bioscience and Biotechnology



Proceedings Book

AUGUST 23-27, 2021

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Editors

Güzin Camkerten Burcu Menekşe Balkan Ilker Camkerten

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Dear Scientist,

The fifth International Congress on Advances in Bioscience and Biotechnology (icabb) was organized in Sarajevo, Bosnia and Herzegovina. We are very happy for organizing this congress in such a beautiful city and country that we have strong historical ties.

We wanted to make this conference little bit special by bringing scientist together from different disciplines of veterinary area and to open new research and cooperation fields for them. In this sense, we desired to bring the distinguished scientist together to get know each other and to develop and implement new joint projects.

The scientist joined the congress was from different country and mostly from Turkey. Total over the one hundred scientists were registered in the congress. The total number of submissions were 35 and after a careful evaluation 24 submissions were accepted by our scientific committee and 2 of them were accepted as poster presentation and 22 of them were accepted as oral presentation and all those presentations was taken place in the conference booklet.

We would like to send our special thanks to the International University of Sarajevo, Universiti Teknologi Malaysia, and Prof Hesham El Enshasy for their contributions. Also, we would like to express our special thanks to the organization team especially Mr. Musa Köse and Mr. İsmet Uzun, ZENITH Group workers, and the scientific committee. And finally, most importantly we thank all the participants individually to join this conference.

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INVITED SPEAKERS

Modern Nutrition: Advances in Systemic Biology as Current (Bio)Technological Approaches

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Introduction. The term of "modern nutrition" is currently almost equal to antiquity of the old testaments: "the food is medication and medication is food". And nowadays a lot of researchers are fascinated with concept of the "Healthy nutrition". Moreover, related to "microbiome" and omics studies suggest that this healthy nutrition suppose must need the individual requirements. Taking also into account the systemic biology and P4 novel medicine strategies visions we need to find a way of the integration and harmonization all our multifaceted knowledge in order to describe sufficiently and properly define the term of "personalised and healthy" nutrition – or by other words "modern nutrition"

Modern nutrition is a multidisciplinary and complex subject combining the approaches of the epidemiology, biochemistry, chemistry, behavioral science, biology, food science and medicine. Thus, we need to use all the available complementary data in order to construct "next generation functional foods".

Aim. The demands to these foods one might list: 1) be natural of origin, 2) be safe, 3) contains minimal or zero chemical / genetically modified additives, 4) be simple in preparation with minimal cooking efforts, 5) be fermented in order to be rich on biological active compounds and beneficial microbes 6) be prepared from naturally cultivated edible plants, 7) be clearly labeled of all the ingredients and 8) better be recognized by brand or at least by codes of food composition data bases.

Ideally will be to have a data about these products exact influence on human health, based on the results of clinical approbation: 1) of their different components or 2) whole meal / diets.

To meet this goal from first glance complicated (bio)technologies should be exploited. Interestingly that the majorities of these requirements are typical for the traditional well-known ethnical dishes that are 1) mostly accepted by nations, 2) can be easily prepared, 3) imagined as food with healthy impact on human health.

The challenges to make these new generation foods widely used are 1) short shelf life, 2) packing and transportation' difficulties, 3) varieties of recipes 4) (bio)technological gaps.

Results and Discussion. Recently we developed a line of such a novel traditional functional modern foods of new generation EdiensTM. These products had been recently created particularly with unique microbial starters which are sequenced and preliminary selected / investigated in numerous in vitro, in vivo, ex vivo models.

Recently we developed a line of such a novel traditional functional modern foods of new generation EdiensTM. These products had been recently created

particularly with unique microbial starters which are sequenced and preliminary selected / investigated in numerous in vitro, in vivo, ex vivo models.

In addition, the synergetic properties of these strains of different phylogenetic groups with most popularly used plant originated biological active compounds isolated from local edible plants and berries had been carefully detected.

Person-specific efficacy to regulate human gut microbiome had been detected for individually prescribed foods via limited controlling diet studies.

Conclusion. In order to promote the implementation of newly developed functional foods relevant databases and AL for the calculation of personalized nutrition needs had been established and proposed.

Key words: Modern Nutrition, Food Composition Data Bases, Functional Food, Individual Nutrition, Human Microbiome, Databases, Al

This work particularly supported by funds of Ministry of Education and Sciences, Grant Agreement No UA 0117U000379 / UA 0120U102244

Climate Adaptation and Marine Biodiversity

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Climate change is the phenomenon of changing weather patterns and the inevitable increase of temperatures globally. While humans are primarily to blame for such a phenomenon, it will harm us and cause significant harm to every living creature on the planet. Climate change has and will directly negatively affect Biodiversity, which measures the number of species present in an area or globally and is a clear indicator of environmental health. It has been observed that increasing temperatures and altered weather conditions have been harmful to almost every organism. Thus, they are endangered, expected to decline in numbers and possibly become extinct. Unfortunately, the effects of climate change have already begun. The only thing we can do is collectively work together to help ourselves and our environment adapts to the expected changes. The key to doing so is innovation, science, and hard work. Biotechnology can allow us to make such adaptation measures and help us save our environment and its inhabitants. Many branches of this vast field of science exist, but genetics, particularly, has proven to be extremely valuable to biodiversity conservation. This particular field is promising because it deals with keeping organisms, or at least tissue fragments from them, in order to preserve them and keep their genetic material available for production in better survival circumstances to eliminate the risk of them going extinct. Biodiversity is a little talked about subject, its value severely underrated, and its true potential is yet to be unlocked. If we can save the remaining species, we open an unimaginable number of resources, pharmaceutical materials, biofuels, and much more goodness that can only be provided with a healthy, balanced environment.

Reverse Vaccinology: The Potential of Genome-Based Antigen Discovery and Vaccine Development Yaqoub ASHHAB

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Generally, vaccines are made of either killed or inactivated microorganisms. This traditional approach includes the growing of microorganisms, a laborious, expensive, and potentially hazardous process. With the advent of genome sequencing technologies, a novel approach for vaccine development has emerged, known as Reverse Vaccinology (RV). The principle of RV is to use the whole-genome sequence(s) of any microbe, including bacteria, parasites, or viruses, to identify the most potential vaccine candidate antigens without the need for cultivating the pathogen of interest. Identifying the candidate antigens is carried out by analyzing the genomic sequences in silico, using specialized computational methods and tools. The in silico discovery phase is typically followed by cloning, expressing, and evaluating the immune response of the shortlisted candidate antigens using in vitro and in vivo assays. In this talk, the basic principles of the original version of RV, which was developed almost two decades ago by Rino Rappuoli and co-workers to design a vaccine against serogroup B meningococcus, will be introduced. Furthermore, a group of different improvements that have been applied over the original RV design will be presented. In addition, some of the current opportunities and challenges facing biomedical scientists in using RV will be discussed.

Bio-Based Research Initiatives Directed Towards Building an African Bioeconomy

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A bio-economy covers all sectors and systems that are reliant on biological resources and their functions and is a new model for industry and the economy. It also exploits the untapped potential stored within millions of tons of biological waste and residual materials. The transition from a fossil fuel-based to a bio-based economy is expected to reduce the dependency on fossil fuels and achieve more sustainability as well as contribute to climate and environmental protection. In recent years, the bio-economy has also become a key focus of political and technological interest in various countries. Similarly, the vision for South Africa's bio-economy is to be a significant contributor to the country's economy by 2030 in terms of the gross domestic product (GDP). In order to achieve this, significant innovation is required for the development of novel industries that generate bio-based services and products. On a macro-economic and developmental level South Africa's thriving bio-economy has the potential to make the country more competitive internationally specially in the industrial and agricultural sectors. More importantly, a stable bio-economy will create more sustainable jobs, enhance food security and creates a greener economy as the country shifts towards a low-carbon economy. At the Council for Industrial Research (CSIR) a strategic research approach focusing on bio-based technology and product development has been adopted. Key platforms (Bio-conversions, Bio-based Products and Bio-manufacturing) have been established to fast track commercialisation and industrial uptake or for the establishment of new Biotech start-ups. An overview of these initiatives and successes will be presented.

ORAL PRESENTATIONS

The Impact of Facile Formulation of Alcohol-based Hand Sanitizer Containing *Eucheuma cottonii* Seaweed on its Sensory Properties and Acceptability

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Seaweed is a form of algae that be obtained abundantly from the sea. This rocky shoreline-growing alga is commonly eaten by the Asian people or used in culinary purposes. To further enhance the economic value of the seaweed, the applications need *cottonii* seaweed and to evaluate the sensory properties and overall acceptability. Different seaweed concentrations (1, 2 and 5% w/v) were screened for their suitability in the alcohol-based hand sanitizer formulation. Antimicrobial testing was also carried out on four common bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida utilis) using Kirby-Bauer disk-diffusion method. A number of 30 volunteers consisting of men and women aged between 19-50 years old were given several samples of hand sanitizers including the commercial hand sanitizer and their evaluations were recorded and analyzed. It was observed that at higher seaweed concentration, the formulation becomes less viscous. The antimicrobial testing of the seaweed hand sanitizer formulation shows significant inhibition zones for all the four microbes tested to be diversified into high-value products. Seaweed have previously proven to have several properties including antimicrobial, thickening and moisturizing properties. With regard to the Covid-19 pandemic attacking the whole world, this study aims to develop a facile formulation of alcohol-based hand sanitizer containing Eucheuma with E. coli having the largest effect. Based on the overall acceptability, the seaweed formulation garnered mean score above 7 (like moderately) which indicates that the developed seaweed hand sanitizer has the potential to be accepted and commercialized.

Keywords: Eucheuma cottonii, seaweed, hand sanitizer, antimicrobial

Green technology influences the chemical composition of *Garcinia atroviridis* Extracts

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Many studies have focused on developing efficient and selective methods for extracting and isolating bioactive natural compounds. The value and quality of natural products in various applications has sparked interest in medicinal plant processing characteristics. This study aimed to improve the extraction of Garcinia atroviridis and characterise the chemical composition at different extraction times. Prior to the pre-treatment process, Garcinia atroviridis was extracted using a maceration method at intervals of 1 hour, 5 hours, 7 hours, and 9 hours. In this work, chemical quality control of the herbal extract was conducted by measuring polysaccharides, glycosaponin and protein. The results showed that the highest yield of extraction (35.2%) was obtained at 3 hours of extraction time. In this work, polysaccharide content was evaluated, and 0.37 % was recovered at the maximum extraction time of 5 hours. However, at 2 hours of extraction time, a greater protein content of 4.47 % was obtained, and at 9 hours of extraction time, a higher glycosaponin content of 62.2 % was gained. As a result, this maceration approach is a promising sustainable approach to enhance the potential of Garcinia atroviridis extract for future food and cosmetic applications.

Keywords: Garcinia atroviridis, maceration extraction, chemical composition

This research work is supported by HICOE Grant (4J272), Ministry of Higher Education Malaysia

In vitro performance of fatty acid-lipid nanocarrier as 5α-reductase inhibitors type II for hair growth promotion

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Androgenic alopecia (AGA) is one of the most common hair loss problems that affect both men and women during their lifetime. Due to the systematic side effects of oral finasteride, there is an increased interest in finding an alternative compound that can reduce hair loss problem but with fewer side-effects. Previous studies have shown that some plant extracts could inhibit 5α -reductase type II (5α R2) and due to the presence of fatty acids. In this study, fatty acid such as oleic and lauric acid were formulated into lipid nanocarrier (FA-LNC) for hair care cosmetic ingredients. However, up to now no specific fatty acid have been tested in vitro on human hair follicle dermal papilla cells to show cell proliferation and inhibition of 5αR2 activity. This research compares the effects lauric acid, C12 and oleic acid, C18:1 and also the performance of FA-LNC on the proliferation of hair follicle dermal papilla cells (HFDPc) and the inhibition the 5aR2 in vitro. FA-LNC with hydrodynamic diameter was found to be 230.1 \pm 27.0 nm with a narrow PDI (0.270 \pm 0.042). Oleic and lauric acid showed no cytotoxicity to the cell even at 1 mg/mL. At 0.0625 mg/mL, oleic acid showed significantly higher proliferation of the hair follicle dermal papilla cells compared to lauric acid (p<0.05). At 6.25%, FA-LNC showed better proliferation when compared to finasteride (positive control, 100 µM), oleic and lauric acid alone (1 mg/mL). In terms of the 5α R2 inhibition, at 0.0018 mg/mL, both fatty acids showed inhibition with no significant difference (p>0.05). Meanwhile, FA-LNC sample showed higher reduction activity compared to finasteride (p<0.05) with no significant difference with oleic and lauric acid alone (p>0.05). This study infers that FA-LNC as plant derived 5α-reductase inhibitors can act synergistically on the cell proliferation and have the potential to be added into hair growth products to inhibit the 5aR2.

Keywords: Androgenic alopecia, fatty acid, human hair follicle dermal papilla cells, $5\alpha R2$ activity

This study is supported by HICOE-IBD UTM (R.J130000.7809.4J317) and University Industry Research Laboratory UTM

New strategies for establishment of beneficial soil microbiological methods under ISO 17025

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Soil-beneficial microorganisms are important for biogeochemical cycles and have been used in agriculture production for decades. In the past years, agricultural practices have failed to promote healthy populations of microorganisms in soil, thus limiting the production yields and threatening sustainability. In this context, adequate monitoring of soil quality by a laboratory using analysis is needed as an indicator for the evaluation of soil quality. However, monitoring and evaluation can only be achieved if analytical methods are subject to an appropriate validation or audit process. The use of a verified method has become a prerequisite for a service laboratory certified to ISO 17025 (International Organization for Standardization) or other regulatory bodies in the country. In the framework of validation methods, the aim of this work is critically to discuss usual concept of regulatory guidelines used and common checklist that can be followed during the verification and validation work. Since nitrifying bacteria have been more prominent among beneficial microorganism analytical applications, the verification was done in accordance with standard method APHA-9245B by assessing the sensitivity, trueness, precision and robustness value of soil, wastewater and fertilizer samples. The method performance of sample analysis is well suited and was acceptable by the guideline with RSD value \leq 0.1, coefficient of variation \leq 10%, trainees value ranges from 60. 67%-127. 27% and robustness value 80-120% of both ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Further study needs to carry out in the validation of an alternative methods to demonstrate its capacity to perform as well as the gold standard reference method.

Keywords: Nitrifying, APHA 9245B, Soil, wastewater, fertilizer

This study is sponsor by bionexus partner Bioeconomy Corporation

Bioprocess optimization for pleuran production by Pleurotus ostreatus using different cultivation strategies.

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Nowadays, large scale mushroom cultivation especially Pleurotus sp. takes attention of many pharmaceutical industries around the world due to their rich and variety of medicinal compound. Extensive research had been conducted to isolate, characterize and produce the importance bioactive compounds from this mushroom. Pleuran (β -(1,3/1,6)-D-glucan), is one of the important compounds belong to glucan group produced by Pleurotus ostreatus. This mushroom can be cultivated using submerged fermentation as a better alternative than the conventional method which used solid state fermentation. However, the production of this polysaccharide using submerged cultivation posed certain challenges due to its low yield. Therefore, this study aim to bioprocess conditions for high production insoluble optimize the of exopolysaccharide pleuran using submerged fermentation. Several media formulations were screened pleuran production. The best medium produced highest of 2.13 gL-1. Subsequently, the selected medium was optimezed using OFAT and statistical approached. By using statistical method, 3.65 gL-1 of pleuran was produced and this is higher compared to the OFAT method which produced only 3.33 gL-1 of pleuran. The optimized medium using statistical optimization was used in batch cultivation. The maximum pleuran production in batch cultivation was slightly higher than shake flask level at 3.75 gL-1. In fed-batch cultivation, full medium feeding was found to produce higher pleuran production of 4.5 gL-1 compared to mono carbon feeding with glucose produced 3.8 gL-1 of pleuran. In conclusion, this study has proposed a reliable approach for high production of plueran by Pleurotus ostreatus using the newly design medium and efficient cultivation strategy in the bioreactor.

Keywords: Bioprocess, submerged fermentation, fed-batch cultivation, pleuran

Study on bio accessibility and release behaviours of Gallic acid and Eurycomanone in herbal supplements products

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Herbal supplement product is a combination of the herbs with other ingredients has a variety of uses which allow them to bind together, regulate the release of the active compound and also upgrade the taste or hide the bitter taste. Bio accessibility is fraction or compound of active ingredients released from its matrix for intestinal absorption which will give impact on their bioavailability. Media solution and time are two factors which affect the behaviour of release of active ingredients. The objective of this study is to evaluate the release behaviour of the herbal supplements products submitted to different media using dissolution testing apparatus. In order to compare the release behaviour, four types of different media; 0.1 M HCl, 30% EtOH, acetate buffer (pH 4.8) and phosphate buffer pH 6.8 were used and samples were collected at 15 minutes time interval for duration of 105 minutes. The dissolution activity of the three brands of both Kacip Fatimah (I, II, III) and Tongkat Ali (TA, TB, TC) were compared. The aliquots sampling and active ingredients namely Galic acid and Eurycomanone in all samples were quantified using a reverse-phase HPLC. Pure standard of Kacip Fatimah (V) and Tongkat Ali (TS) powder were used as a benchmark. Results obtained indicate that the dissolution efficiency for active ingredients for all brands were varied, however amongst those, sample labelled as I in Kacip Fatimah and sample labelled as TA in Tongkat Ali showed the highest release rate of amount of galic acid and eurycomanone in an acidic medium (0.1 M HCl), respectively. Meanwhile, sample labelled as TC showed the lowest released rate of Eurycomanone in all medium tested. HPLC results showed that content of the Gallic acid in sample labelled as I was the highest while for content of Eurycomanone in sample labelled as TA was the highest. Complex mixture of ingredients which added in the Kacip Fatimah and Tongkat Ali products may give impact to the release behaviour of the active ingredients and affected by the solution media used.

Keywords: Herbal mixture, dissolution parameter, HPLC, quality

Extraction and Characterization of Astaxanthin From the Waste of Deep Water Pink Shrimp (Parapenaeus longirostris) Obtained from Iskenderun Bay

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The aim of this study is to purpose a simple and effective method for the isolation of high-value pigment astaxanthin from shrimp waste, a low-value raw material. In this study, acetone was used as an extraction solvent. Mobile phase for TLC was acetone: +hexane in the ratio 3:7 (v/v). The samples used in the study belong to the *Parapenaeus longirostris* species and were obtained from local fishermen operating in the Iskenderun Bay / Turkey. Shrimp waste was transported to the laboratory in a sterile container filled with ice. The wastes used in shrimp are the cephalothorax, abdominal shell and tail portion. Adhering meat from the cephalothorax was removed and the waste was washed under water and samples were dried at 50 °C with drying oven. Packed in polyethylene bags and stored at -18 °C until use. In this study, astaxanthin was extracted from shrimp shell waste using organic solvents (petroleum ether and acetone). Characterization of Astaxanthin pigment was performed with TLC buy comparing the Retardation Factor (Rf) as indicated in the Lorenz Todd standard chromatogram, in which three bands of Astaxanthin (Rf=0.36), Astaxanthin monoester (Rf=0.60) and Astaxanthin diester (Rf=0.75) were detected.

Keywords: Astaxanthin, Parapenaeus longirostris, thin layer chromotography

Assessment of Asthma-Associated miRNAs in Children with Rhinovirus Respiratory Infections

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Aim: Asthma is one of the major health problems all over the world. In fact, approximately two-thirds of asthma exacerbations are associated with human rhinovirus (HRV). MicroRNAs (miRNAs), small single-stranded, non-coding RNA molecules which are 22-25 nucleotides in length, are produced in cells. It has been reported that miRNA expression levels may be associated with the pathogenesis of diseases through the regulation of the production of target proteins. Expression changes of several miRNAs have also been found to play a role in the development and/or improvement of asthma. Still, relatively little is known about the role of miRNAs in asthma. We aimed to investigate serum levels of five asthma-associated miRNAs in children with lower respiratory tract infection (LRTI) caused by HRV compared to age-matched healthy controls.

Materials and Method: Serum samples were collected from the 37 pediatric patients, aged 0-3 years, with LRTI proven to be caused by HRV using RT-PCR assay (FTD Respiratory pathogens 21, Fast Track Diagnostics, Luxembourg) and from the 30 agematched healthy controls. miRNA let-7a-5p, miR-9-5p, and miR-155-5p were investigated from the serum samples by using RT-PCR assay (RTA miRNA Kit, Kocaeli, Turkey). miRNA serum expression levels were analyzed by the 2($-\Delta\Delta$ CT) method.

Results: In the present study, we found that let-7a-5p, miR-9-5p and miR-155-5p levels were significantly increased, while miR-146a-5p and miR-19-3p levels were significantly decreased in the sera of children with HRV infection compared to the age-matched control group. Assessment of serum expression changes of asthma-associated miRNAs in children with respiratory tract infections caused by HRV is important for understanding the effect of HRV on the pathogenesis of asthma through miRNA expression changes. On the other hand, longitudinal studies based on clinical observation are needed to determine the association of HRV infection with the development of asthma and miRNA-induced epigenetic effects."

Keywords: Human rhinovirus, asthma, miRNA let-7a-5p, miR-9-5p, miR-146a-5p, miR-19-3p, miR-155-5p

This study is supported by Scientific Activities Support Program of Necmettin Erbakan University

Oral Presentation

The Investigation of the Therapeutic Effects of Epigallocatechin-3-Gallate Against Cisplatin-Induced Liver Damage in Rats

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In this study, it is aimed to investigate the possible protective effects of epigallocatechin-3 gallate (EGCG) in rats exposed liver damage with cisplatin. The animal experiments part of this study was conducted in the F.U Experimental Animal Research Center (FUDAM) with the permission of the F.U Animal Experiments Ethics Committee dated 17.02.2021 and numbered 2021/03. In the study, 28 Wistar albino male rats (n = 28, 8 weeks old) were divided into 4 groups and each group included 7 rats. Groups: (i) Control Group: Standard diet, (ii) EGCG Group: Standard diet + EGCG (50 mg/kg CA, ip), (iii) Cisplatin (CP) Group: Standard diet + Cisplatin (CP) (7 mg/kg CA), (iv) EGCG + Cisplatin (CP) Group: Standard diet + Cisplatin (CP) (7 mg/kg CA) + EGCG (50 mg/kg CA, ip). The rats were decapitated after 4 weeks and their liver tissues were taken and examined. Expression levels of caspase-3 and Bcl-2 proteins in liver tissue were determined by western blotting technique, lipid peroxidation MDA (malondialdehyde) analysis, catalase and GSH (glutathione) levels were determined by spectrophotometer. Compared to the Bcl-2 protein expression and malondialdehyde (MDA) level decreased, caspase-3 protein expression level, glutathione (GSH) level and catalase activity (CAT) were significantly increased in the groups EGCG + CP compared to the group CP. As a result of this study, it was determined that EGCG treatment has protective and therapeutic effects against liver damage.

Keywords: EGCG, liver injury, cisplatin, MDA

Acknowledgement: This work was supported by Firat University Scientific Research Projects Unit (FUBAP) with FF. 20.04 project number.

The Effect of Fulleren C60 Nanoparticle on Caspase-3, Bcl-2, Nrf-2, NF-κB, TNF-α, Cox-2, p53, IL-6, IL-1α and MAPK Protein Signal Pathways Against Breast Cancer Exposed by DMBA (7,12-Dimethylbenz [a] Anthracene) in Rats

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In this study, the anticancer effect of C60 nanoparticle against breast cancer caused by DMBA (7,12-dimethylbenz [a] anthracene) in Wistar albino female rats was investigated. The animal experiments part of this study was conducted in the F.U Experimental Animal Research Center (FUDAM) with the permission of the F.U Animal Experiments Ethics Committee dated 13.02.2019 and numbered 2019/03. In this study, 60 Wistar albino female rats (n = 60, 8 weeks old) were used. These rats were divided into 4 groups and each group included 15 rats. Groups: (1) Control Group: Fed with standard diet; (2) Group C60: C60 (1.7 mg/kg bw, oral gavage); (3) DMBA Group: DMBA (45 mg/kg bw, oral gavage); (4) C60 and DMBA Group: C60 (1.7 mg/kg bw, oral gavage) and DMBA (45 mg/kg bw, oral gavage) group. The rats were decapitated after 16 weeks and their breast tissues were taken and examined. Expression levels of caspase-3, Bcl-2, Nrf-2, NF-κB, TNF-α, Cox-2, p53, IL-6, IL-1α and MAPK proteins in breast tissue were determined by western blotting technique. As a result, Bcl-2, NF-KB, TNF-a, Cox-2, IL-6, IL-1a and MAPK protein expression levels decreased, caspase-3, Nrf-2 and p53 protein expression levels were significantly increased in the groups C60 + DMBA compared to the group DMBA. According to the results obtained from this study, it suggests that the C60 nanoparticle will make significant contributions to the development of new drugs for breast cancer treatment.

Keywords: Apoptosis, Bcl-2, breast cancer, caspase-3, fulleren C60, Nrf-2, NF-кВ

Acknowledgement: This work was supported by Firat University Scientific Research Projects Unit (FUBAP) with FF. 20.07 project number. In addition, this study was supported by the Council of Higher Education (CoHE) 100/2000 Biotechnology priority field doctoral project and The Scientific and Technological Research Council of Turkey (TUBITAK) 2211/C program.

An investigation on thiol/disulphide homeostasis on cattle naturally infected with bovine ephemeral fever

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Three-day sickness or bovine ephemeral fever is an acute febrile viral disease instead of cattle and ruminant species on Middle East, Asia, Africa and Australia. The aim of this study was to clarify the thiol/disulphide homeostasis on cows naturally infected with bovine ephemeral fever. For this propose total of 45 cows were enrolled to study and divided in to three equal (n=15) groups. Cows were clinically infected, recovered and healthy were determined as Group I, Group II and Group III, respectively. Blood samples were taken without attempting to treat any of the animals in the groups. The native thiol levels were found to be significantly lower in Group I ($262,9 \pm 25,1$) and Group II ($253,1 \pm 39,1$) compared to Group III ($300,8 \pm 52,4$). Non-significantly reduces were observed on Total thiol levels were also detected in Group I ($339,1 \pm 23,9$) and Group II (242,5 ± 40,3) compared to Group III (367,1 ± 55,6). Disulphide levels, disulphide/total thiol, native thiol/ total thiol and disulphide/native thiol ratios were similar in all the groups (P>0.05). In conclusion, bovine ephemeral disease in cows was affected by the thiol disulphide hemostasis. This is the first study to determine the thiol/disulphide hemeostasis in cows with bovine ephemeral fever. Therefore, the results achieved in this research might be ground for further studies to include antioxidants in treatment practices.

Keywords: Thiol/disulphide homeostasis, ephemeral fever, cattle

Corona Virus and Our Environment

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Corona virus disease (COVID 19) is a global pandemic, affecting human life severely. It has infected 181.5 million people world-wide which resulted into death of 3.9 million (WHO 2021). In April and May 2021 sudden surge in cases of corona virus infection was reported from different parts of India. Many scummed to death while those who somehow survived from COVID 19 tsunami has suffered from post-covid symptoms. Considering the scale of its impact, it is important to investigate the public opinion about the spread and origin of corona virus. As COVID is a zoonotic disease we therefore asked public whether there is any linkage between environment and spread of corona virus? To assess the public opinion, we conducted an online survey with the help of undergraduate students of the Shivaji College during June-July, 2021. An online questionnaire on 'Google forms' was prepared and circulated by students through social media and email. A total of 1260 individuals participated in the survey. Based on the survey we found that most of the participants were aware about the causative organism of COVID 19. Majority of them agreed that there is a linkage between degradation of environment and spread of corona virus. They also believe that deforestation, wildlife trade, hunting, landuse change have played role in spread of zoonotic diseases. They further believed that spread of such diseases would likely to aggravate under climate change. Majority of them agreed that vaccination along with conservation of biodiversity, prevention of deforestation landuse change, prohibiting wildlife trade and human wildlife conflict could effectively prevent the spread of zoonotic diseases such a COVID 19. However, around one-fourth of them were of neutral opinion and believed evidences and facts published in print and online media were inconclusive. The current study though presented mixed response from Indian public, however higher proportion of individuals believed a strong linkage between environment and corona virus spread.

Keywords: Corona virus, zoonotic diseases, survey, public perception, India

A Novel Formulation for a Probiotic Supplemented Confectionery with Natural Ingredients

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Probiotics are living organisms that have beneficial effects on host by regulating the microbial balance of the intestinal system. Although mostly in dairy products, it is possible to see examples of food products supplemented by probiotics in bakeries, chocolates and confectioneries. Nowadays, the COVID-19 pandemic that the world suffers increased the demand for such functional food products including probiotics. Due to probiotics having potential effects on strengthening the immune system, a novel formulation for a probiotic supplemented confectionery with natural ingredients is proposed together with a market research to compare the benefits of similar products. The formulation with natural ingredients in the form of jelly beans supplemented by probiotics in the same package suggests varying potential benefits specifically focusing on the probiotics including Lactobacillus species. In this study, a sensory evaluation was carried out to compare the consumer demand for the jelly formulation of the product. 35 untrained panelists were given 4 different samples with varying ratios of carrot and spinach together with a fixed ratio of lemon juice and apple sugar. The panelists were first asked the questions that would determine the general consumption trends and then the parameters including willing to buy, smell, appearance, taste, sweetness, color, texture, shape, bitterness, aftertaste, acidity, overall acceptability were asked to be evaluated in a 7-point scale with 9 questions. Accordingly, an average score of over 4 was achieved with the novel formulation for the confectionery supplemented by probiotics as follows (for 100 g of the product): 31 g of carrot, 7 g of gelatin, 10 g of apple sugar and 0.72 g of lemon juice and 51 g water. These amounts meet 13.2% of protein, 1.23% of carbohydrate, 2.5% of water, 3.3% of sugar, 7.9% of fiber, 11.6% of Vitamins A and C, 0.1% of fat. In addition, the probiotic content reaching over log 7 cfu/mL is suggested to meet 100% of the daily needs. In conclusion, it was depicted that production of a probiotic confectionery containing vegetables or fruits would be useful to help fight several diseases and reach a stronger health level. In the future, this product can be modified for both probiotic bacteria content and flavor in various foods in accordance with consumer feedbacks. The outcomes provide a guide together with the recommendations for potential probiotic research in candy and confectionery industry.

Keywords: Probiotics; confectionery; natural ingredients; lemon juice; carrot

Acknowledgements: The authors gratefully acknowledge Food Engineer Eylül Naz Aktaş for assistance with the sensory analysis.

Ethics approval: Ethics approval and consent to participate he questionnaire and methodology for this study was approved by the Science and Engineering Research Ethics committee of Izmir University of Economics (Ethics approval number: B.30.2.IEOFMB.0.05.05-020-040)

Oral Presentation

Investigation of novel industrial proteins from different source by metagenomic approaches

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The discovery of novel enzymes from nature has attracted attention due to the benefits of enzymes in industrial synthesis technology. Catalysis with enzymes offers several advantages over chemical catalysis. Enzymes improve manufacturing processes, decrease energy usage, work under mild reaction conditions, reduce hazardous byproducts, and increase substrate specificity. Enzymes are also biodegradable thus offer an environmentally friendly technology. There are still many types of microorganisms that are not explored or identified yet, to be a novel enzyme source.

Since less than 1% of total microorganisms in nature can be cultured in the laboratory, a combined approach has been adopted for new protein mining, which includes the functional screening of a microbial resource and a metagenomic library. Metagenomics is a strategy used to analyze whole microbial genomes by extracting total DNA directly from environmental samples without culturing. It is important to discover new proteins that can be used to develop new biotechnological approaches. In our studies, we perform metagenomic studies to determine the microbial community in different environments and to obtain new enzymes. Metagenomic libraries that we obtained from different sources such as rich humus soils, butcher's wastes buried soils, acidic and halophilic food fermentation environments, and unusual cave environments continue to be screened for their industrial potential with sequence-based and functional-based approaches. The determination of microbial diversity in these environments have been completed. Enzymatic activity has been screening according to the culture-dependent and independent approaches. As a result of these studies novel enzymes with glucanase, lipase, and dehydrogenase activity were isolated and recombinantly expressed. Optimization of heterologous expression in different hosts and, biochemical and thermodynamic characterization studies of enzymes in different pH, temperature, salt concentration and organic solvent are in progress."

Keywords: Metagenomics, microbial diversity, novel enzymes, biotechnological production

Effect of dietary taurine supplementation on growth performance of juveniles shibbot (*Tor grypus*)

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This study was conducted to evaluate the effects of taurine on growth performance of Shibbot fish (*Tor grypus*). In the study, 0.00% (C), 0.5% (G1), 1.0% (G2) and 1.5% (G3) taurine were added to the bait of Sabut fish and fed for 60 days. The effects of Taurine on the growth parameters of the Shibbot fish and feed evaluation and aquaculture economy were investigated. Live weight gain (WG), feed intake (FI), Feed conversion rate (FCR), Protein efficiency ratio (PER), Specific growth rate (SGR), Survival rate, Economic conversion rate (ECR) and its effects on the economic profit index (EPI) were investigated. At the end of the study, Sabbut juveniles with an initial weight of 2.28 ± g reached 3.11 ± 0.16 g, 3.05 ± 0.17 g, 3.25 ± 0.11 g, and 3.14 ± 0.26 g respectively. It has been observed that the addition of taurine at the rate of 1% has a positive effect on FCR value in breeding of Shibbot fish and other trails have reached similar and higher FCR values. Considering the contribution of taurine in terms of aquaculture economy, it was also determined that the G2 allows production at a lower cost (USD / kg). It has been observed that adding taurine to Shibbot fish feeds does not contribute to EPI value (economic profit index).

Keywords: Taurine, aquaculture, shibbot fish.

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Extracellular keratinases of *Cladosporium sphaerospermum* for biodegradation

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Animal husbandry produces enormous amounts of by-products that need to be disposed of, e.g., wool, feathers, and bristle. A major structural component of these wastes is keratin - a rigid fibrillar protein with a protection function that is hard to degrade. Enzymatic biodegradation is a beneficial and green approach to keratinwaste disposal. A few organisms groups are capable of producing keratinases enzymes that break down keratin - including micromycetes. For a filamentous fungus Cladosporium sphaerospermum, we showed inducible keratinolytic activity in alkaline conditions in previous studies, but for biotechnological application, a proper study is essential.

For further investigations, submerged fermentation of C. sphaerospermum was performed in a previously selected nutrient medium that differs in keratin sources inducing enzyme synthesis (ground chicken feathers, purified keratin from wool) and pH (8, 9). General proteolytic and keratinolytic activities were determined with culture liquid with 1% suspensions of casein and keratin (prepared on 0.05M Tris-HCl buffer, pH 8.2) respectively and measured spectrophotometrically. One unit (U) of activity was defined as an increase of absorbance by 0.01 under the assay conditions (37 °C).

The highest general proteolytic activity was measured with a sample obtained on the 3-rd day of cultivation on a medium with purified keratin (pH 8), representing 77.3 U. The highest keratinolytic activity was registered after three days of cultivation with ground chicken feathers (pH 9), reaching 45.0 U. Moreover, it remained stable, slightly decreasing to 42.3 U by the seventh day of cultivation.

Thus, for C. sphaerospermum, an intense and stable synthesis of keratinases observed when growing on feathers illustrates the potential of C. sphaerospermum for biodegradation of poultry waste.

Keywords: Keratinases, micromycetes, biotechnology, biodegradation

Histology of Digestive System in the Nannospalax xanthodon (Rodentia: Spalacidae)

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Blind mole rats (Nannospalax xanthodon) are subterranean rodents with various morphological and physiological adaptations to underground life. The aim of the present study was to reveal histological structure of the digestive system of the N. xanthodon which usually prefers a foods such as plant root, tubers and bulbs. For this purpose, five blind mole rat of both sex were used for this study. Tissue samples of the digestive tract were kept in 10% neutral buffered formalin, processed using routine histological methods, and mounted in paraffin blocks. Six micrometer-thick sections were cut and stained with Crossman's trichrome, H&E, alcian blue (pH 2.5) and periodic acid Schiff method.

The results of this study have shown that the esophagus of N. xanthodon is lined by stratified squamous keratinized epithelium with no lamina muscularis and glands. The stomach of this blind mole rat is unilocular compound type. The glandular region contains simple tubular gland, that is lined by mucous neck cells, chief cells and parietal cells. Intestinal glands (crypts) are observed in the lamina propria layer of the duodenum. The length of the villus intestinalis was shortened in the ileum compared to the duodenum, and the number of goblet cells increased considerably. Aggregated lymphoid follicles were observed in the lamina propria and submucosa layers. Tunica muscularis layer was thicker in colon and rectum. Histologically, it was observed that the liver consisted of polygonal shaped lobules. It was determined that the interlobular interstitium was not prominent around the lobules. Some of the polygonal shaped hepatocytes were found to be binucleated. The wall of the gallbladder (vesica fellea) is formed as four layers, inward to outward: lamina epithelialis, lamina propria, tunika muscularis, and serosa. The lumen of the vesica fellea is lined with a high columnar epithelium. Tunica muscularis is a narrow layer of smooth muscle cells and dense collagen and elastic fibers.

In conclusion, although the digestive system of blind mole rat is histologically similar to other rodents, it was observed in the present study that it has also some specific features of its own. These findings are important in having a better understanding of their feeding and adaptation to underground life.

Keywords: Histology, digestive system, Nannospalax xanthodon

Oral Presentation

Using quantile regression approach to evaluate the effects of some selected parameters on daily milk yield in Holstein cows

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Milk yield is an important parameter for the dairy products industry. Days in milk, lactation number and the number of insemination are also reproductive factors that affect milk yield. In the field of veterinary medicine, the effects of these factors (independent variables) on milk yield (dependent variables) can be determined using regression models. The aim of this study was to evaluate days in milk, the number of lactation and the number of insemination that affect the daily milk yield in Holstein cows with quantile regression approach.

The material of the study consisted of the yield records of 112 healthy and multiparious holstein cows. The cows were kept in a private farm and had no dystocia. The effects of days in milk, lactation number and the number of insemination on daily milk yield were examined by linear regression and quantile regression models. First, the distribution of error terms, outliers and influential cases (Mahalanobis distance) were determined for the suitability of linear regression. The quantile regression model, which is an alternative approach to the linear regression method, was applied to determine the parameters that may affect daily milk yield.

As a result of statistical analysis, it was determined that the error terms in linear regression did not fit the normal distribution. Also, there were influential cases according to the Mahalanobis distance. In the quantile regression model, which is a flexible approach compared to linear regression, 0.25th, 0.50th and 0.75th quantiles were examined. It was observed that the number of insemination in 0.25th quantile had an important effect on daily milk yield (p<0.05), while days in milk (p>0.05) and number of lactation (p>0.05) were insignificant. In 0.50th and 0.75th quantiles, the parameters included in the model were not statistically significant.

As a result, the number of insemination had a effect on daily milk yield in only 0.25th quantile. In addition, the quantile regression model can be used as an alternative method when the assumptions are not met in linear regression.

Keywords: Milk yield; quantile regression; reproductive parameters

Determination of β -Glucosidase deficiency by using in house ELISA model

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Rare diseases (RD) refer to diseases seen less than 1:2000. There are about 8000 species of RD of which 80% are of genetic origin. In recent years, lysosomal storage diseases, which belongs to RD family, (LSD; most commonly seen species such as Gaucher) are of enhanced importance all over the world, seem a lack of a specific enzyme/lysosomal component in the body. According to the Health Implementation Communiqué, these diseases are diagnosed according to the measurement of enzyme level or mutation analysis results are compatible with the RD.

In this study; we tested novel conjugate systems to being developed the sensitivity of antibodies for β -Glucosidase level by using sandwich ELISA method. We coated the plates with monoclonal anti-GBA (1 µg/mL in Glycerol 40%) and Anti-GBA antibody (0,5 µg/mL in Glycerol 40%). Then we compared free conjugate and hybrid conjugates. We tested different concentration (1,56-100 ng/mL) of GBA enzyme standards with covered plates. We accepted the cut-off value between the highest value of the negative controls and the lowest value of the positive controls (2 x mean of negatives). In that sense, only the formulas correctly discriminated amongst the positive and the negative controls. Our study evaluated the diagnostic accuracy of β -Glucosidase protein level. This aims to eliminate and alleviate the socio-economic burdens of RD, to diagnose easily and correctly, transition to rapid treatment or to develop information production and methods that can be effective in preventing diseases.

Keywords: ELISA, Gaucher Disease, diagnostic System

This study is supported by Scientific Research Projects Committee of Nevsehir Haci Bektas Veli University (Project No: BBAP20F14) **POSTER PRESENTATIONS**

Vermiculite as a new carrier for fungal proteases production in solidstate fermentation conditions

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Micromycetes are known as producers of extracellular proteases with broad substrate specificity. An efficient approach to increase the hydrolases yield for biotechnology is using solid-state fermentation (SSF). SSF provides overproduction of enzymes because fungal mycelium penetrates and entwines the carrier particles, imitating natural growth conditions. Besides, some enzymes are synthesised only during solid-state but not submerged fermentation. Therefore, carrier selection plays an essential role in enzyme production for industrial applications.

As high-active protease producers, Aspergillus ochraceus L-1 and Aspergillus ustus 1 were selected for the study. Strains were cultivated stationary at 28 °C on polyurethane foam, perlite, silica gel, and vermiculite, moisturized with a previously selected nutrition medium. Proteolytic enzymes were eluted on the 5-th day of cultivation with 0.05 M Tris-HCl buffer, pH 8.2. The general proteolytic and fibrinolytic activities were determined using 1% suspensions of casein and bovine fibrin. Proteolysis was carried out with eluted enzymes and measured spectrophotometrically. The activity was expressed in µmoles of tyrosine formed in 1 min in 1 mL of culture liquid and was calculated using the calibration curve. For productivity, the obtained result was divided by the mass of dry mycelium, previously determined gravimetrically.

Compared with silica gel, the yield of proteinases on vermiculite was 3 times higher than other carriers - from 7 to 18 times. This proved polyurethane foam and perlite as ineffective carriers for SSF. Proteolytic activity of both micromycetes was the highest when growing on vermiculite (52.4 and 57.2 U/mg of biomass 10^-3 for A. ochraceus L-1 and A. ustus 1, respectively). Also, for A. ustus 1, SSF induces the production of fibrinolytic proteases, with the highest activity (224.4 U/mL) observed with vermiculite.

Thus, vermiculite may be considered a suitable carrier for SSF, increasing producers' productivity and yield of enzymes for biotechnology.

Keywords: Solid-State Fermentation, Vermiculite, Proteases

Poster Presentation

Effects of mitochondrial-derived peptide (mots-c) on cell death and dna damage of mcf-7 breast cancer cells

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Breast cancer is an important health problem among women. The discovery of new therapeutics to be used in cancer treatment is important for increasing success in treatment. Mitochondrial-derived peptide (MOTS-c) is encoded by mitochondrial DNA and is 16 amino acids long. MOTS-c is involved in cellular energy metabolism by activating 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)-mediated AMP-activated protein kinase (AMPK).

The present study aimed to determine the cytotoxic and genotoxic effects of MOTS-c on the human breast cancer cell line, MCF-7.

In the present study, MCF-7 cell line was used and the cells were cultured with DMEM medium. After treatment with MOTS-c and the standard anti-cancer drug 5-fluorouracil (5-FU), the cytotoxicity level in the cells was determined by MTT assay, and the genotoxicity level was determined by Comet assay. Furthermore, mRNA and protein expression levels of genes involved in cell death processes were investigated using qPCR and Western Blot, respectively.

The results showed the cytotoxic effect of MOTS-c on MCF-7 cells was more pronounced at 72 hours. In Comet analysis of MCF-7 cells after MOTS-c application, tail intensity (TI), tail lenght (TL), and tail moment (TM) parameters were significantly increased compared to control group (p<0.05). MOTS-c application caused a significant decrease in Akt and Bcl-2, while an increase in AMPK, Beclin1, p53, TSC2, and ULK1 expressions in MCF-7 cell line(p<0.05).

These results are the first data showing that MOTS-c causes cell death through DNA damage in MCF-7 cells. In conclusion, MOTS-c may cause an increase in AMPK-mediated TSC2 and ULK1 gene expressions and thus can activate the autophagy pathway in cancer cells.

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Keywords: 5-Fluorouracil, Apoptosis, Cytotoxicity, Genotoxicity, MCF-7, MOTS-C

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