

# BioVision Alexandria

New Life Sciences: Towards SDGs

20–22 April 2018

**BioVision Alexandria 2018**

Poster Session Abstracts

# BioVisionAlexandria 2018

## Poster Session Abstracts

The abstracts are presented in alphabetical order by the presenter's last name.

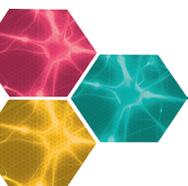


# **Detection of Soil Salinity for Bare and Cultivated Lands Using Landsat ETM + Imagery Data**

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Soil salinization is a standout amongst the most basic environmental universal issues due to its adverse effects on agricultural productivity and sustainable development. Remote sensing is an important tool for investigating soil characteristics such as soil salinity. In saline soils, the spectral reflectance of salt at the surface or of vegetation execution that was adversely influenced by salt varies with different salinity levels. Therefore, many salinity and vegetation indices have been developed and used. This study used ground data and Landsat Enhanced Thematic Mapper Plus (ETM+) satellite images (visible and near-infrared reflectance) to compare between eleven spectral indices, which encompassed soil salinity and vegetation indices, to determine the best index to the estimations of soil salinity for bare and cultivated soil. Soil samples were gathered from two locations in Beheira Governorate in Egypt; 24 samples from Wadi El Natron (bare soil) and 22 samples from El Bostan (cultivated soil) and the soil samples locations were overlaid on ETM+ satellite image to extract the exact index values. The Electrical Conductivity (EC) measured in saturated soil-paste extract. Among those spectral indices, SI3 showed the highest correlation coefficient with EC ( $R^2 = 0.77$ ) according to linear regression analysis and S6 according to Polynomial regression ( $R^2 = 0.83$ ), followed by S3 for bare soil. NDVI and SAVI get the best result for assessing the soil salinity of cultivated soil ( $R^2 = 0.83$  and  $0.76$ ) according to Polynomial and linear regression, respectively, followed by RVI.



## Antibacterial and Wound Healing Evaluation of Cinnamyl Gelatin Membranes

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For the ideal wound dressing materials, it should provide proper physical, mechanical and biological properties to inhibit secondary infection. Furthermore, supply excellent physiological environment to facilitate cell adhesion, proliferation and/or differentiation. The aim of this study was to develop and characterize cinnamyl gelatin wound dressing membranes and investigate its wound healing ability *in vitro*. The prepared membranes were characterized using tensile strength, UV-Vis Spectroscopic analysis, Thermal Gravimetric Analysis (TGA), Differential Scanning Calorimeter (DSC), and Scanning Electron Microscope (SEM). The *in vitro* analysis was done including; antibacterial evaluation against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus haemolyticus* and *Klebsiella pneumoniae*; the cytotoxicity of the membranes toward human Peripheral Blood Mononuclear Cells (PBMC) and blood compatibility using hemocompatibility and thrombogenicity tests. The obtained results suggest that cinnamyl gelatin membranes are promising materials for wound healing applications.

Keywords: Cinnamyl gelatin; Wound healing; Membranes; Antibacterial; Bioevaluation.

## Micropropagation and Start Codon Targeted Characterization of Four Stevia Cultivars in Egypt

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*Stevia rebaudiana Bertoni* is a natural low-calorie crop and commercially used as a non-caloric sweetener for diabetic patients. It is also used as cosmetic ingredient, pickling agent, and dentifrice. Four cultivars (Spantia, Shou2A3, China, and High Sugar) of stevia were included to optimize *in vitro* micropropagation. Four different combinations of hormonal treatments were investigated [6-benzylamino purine (BAP) + Kinetin (Kin) (0.25 + 0.25 mg/l); Forchloefenuron (Cpu) + Kin (0.25 + 0.25 mg/l); Cpu+ Kin (0.5 + 0.25 mg/l); and the control medium (hormone-free)]. Out of the different media components, the hormone-free medium produced the best performance of explants. The analysis of variance showed that the control treatment was the most significant for all traits except the number of branches per cutting. Hardening of rooted plants was performed in plastic pots with 70% survival percentage during acclimatization. Molecular characterization, of the four stevia cultivars, was conducted using 11 SCoT primers. The SCoT analysis resulted in 122 amplicons, of which, 62 amplicons (51%) were polymorphic. The range of polymorphism was between 6% and 91%. The range of polymorphic amplicons per primer was between one and 12 amplicons. The SCoT-16 produced the highest number of polymorphic bands (12). Meanwhile, the SCoT-24 produced the least polymorphism (6%). The current study provides a new micropropagation system with low cost, high efficiency, and hormone-free application. Additionally, the study provides the first molecular characterization of stevia using SCoT marker system. Finally, SCoT markers associated with cultivars having high and low contents of stevioside can further be validated by marker-assisted breeding studies.

## Development of High-Yield Specific Markers, Gene Mining and Early Diagnostic Tool in Jojoba

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Jojoba (*Simmondsia chinensis*) is a long-lived, which produce seeds contain high quantity of unique oil reaches 50–55% which knew as liquid wax ester. There is a high variability among jojoba trees as some trees give high yields and others give low yield. Accordingly, there is a crucial need to know the genetic factors that are characteristic for the high yield trait in jojoba. Start codon targeted polymorphism “SCoT” is a gene targeted marker technique based on the polymerase chain reaction. In our study, out of 30 SCoT primers were used, only four SCoT primers revealed differential bands between high and low yield groups acting as specific markers for high yield only. In order to identify the isolated markers ‘Gene-Mining’ is performed, which is categorized at two levels: gene structure and gene function. Our results showed that, the four sequenced markers specific for high yield jojoba trees are coding for the following genes: Putative Recombination initiation Defect (PRD1), Xyloglucan endotransglucosylase/hydrolase protein 22 (XTH22), heavy metal-associated isoprenylated plant protein 3-like (HIP3), and Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL). By the aid of modern technology a diagnostic tool “Strip assay” is developed to identify the early stage superior jojoba clones through detecting the strains which carry the DNA sequence specific for the high yield trait. It is observed that in high yield jojoba strains there are a band appears due to the hybridization of probe with high yield specific sequence when there are no band appears with low yield jojoba strains.

## **D-Galactose Induced Cardiac Aging: Effect of *Dunaliella Salina* and its isolated Beta-Carotene**

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Aging is a natural process that is associated with marked alterations in the structure and/or function of the heart. This study aimed to investigate the possible protective role of biomass of *Dunaliella salina*, its polar and carotenoid fractions as well as its isolated  $\beta$ -carotene on cardiac dysfunction associated with D-galactose (D-GAL). Aging associated cardiac dysfunction was induced in rats by injecting of D-GAL (200 mg/kg; I.P) for 8 weeks. D-GAL injected rats were treated with two regimens; where biomass extract of *D. salina*, its polar and non-polar extracts were given orally for two weeks during D-GAL injection in the protection regimen or biomass extract of *D. salina*, its polar and carotenoid fractions as well as its isolated  $\beta$ -carotene were given orally for 28 consecutive days after the D-GAL injection in the treatment regimen. The results of the study showed that D-GAL injection for 8 weeks is accompanied with dramatic electrocardiographic (ECG) changes as well as profound elevation in serum levels of homocysteine, creatinine kinase isoenzyme and lactate dehydrogenase in addition to reduction the cardiac contents of glucose transporter 4 (GLUT-4). D-GAL also induced a reduction in cardiac superoxide dismutase activity and elevation of inducible nitric oxide synthetase and interleukin-6. On the other hand, oral administration of the biomass extract of *D. salina*, its polar and carotenoid fractions as well as its isolated  $\beta$ -carotene attenuated the D-GAL-induced disturbances in the above mentioned parameters. Finally, the histopathological examination emphasized the obtained results. In conclusion, *D. salina* and its isolated beta-carotene content ameliorate D-GAL-induced aging associated cardiac dysfunction which may be through GLUT-4 activation and its potent antioxidant activity.

## Developing Transgenic Wheat to Facing Rusts and Powdery Mildew by Chi26 Gene for Fungal Resistance

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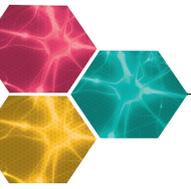
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The main aim of this study was to improve the fungal resistance in bread wheat via transgenesis. Transgenic wheat plants harboring barley chitinase (chi26) gene, driven by maize ubiquitin promoter, were obtained using biolistic bombardment, whereas the herbicide resistance gene and bar, driven by the CaMV 35S promoter was used as a selectable marker.

*Results:* Molecular analysis confirmed the integration, copy number, and the level of expression of the chi26 gene in four independent transgenic events. Chitinase enzyme activity was detected using a standard enzymatic assay. The expression levels of chi26 gene in the different transgenic lines, compared to their respective controls, were determined using qRT-PCR. The transgene was silenced in some transgenic families across generations. Gene silencing in the present study seemed to be random and irreversible. The homozygous transgenic plants of T4, T5, T6, T8, and T9 generations were tested in the field for five growing seasons to evaluate their resistance against rusts and powdery mildew. The results indicated high chitinase activity at T0 and high transgene expression levels in few transgenic families. This resulted in high resistance against wheat rusts and powdery mildew under field conditions. It was indicated by proximate and chemical analyses that one of the transgenic families and the non-transgenic line were substantially equivalent.

*Conclusion:* Transgenic wheat with barley chi26 was found to be resistant even after five generations under artificial fungal infection conditions. One transgenic line was proved to be substantially equivalent as compared to the non-transgenic control.

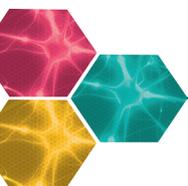


## **Value of Endothelial Progenitor Cells as Novel Markers for Tumor Angiogenesis in Breast Cancer**

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Early detection of breast cancer can reduce the morbidity and mortality of breast cancer occult at the time of primary diagnosis. The identification of markers to distinguish between normal cells, tumorigenic cells and different stages of breast cancer is of critical importance for diagnosis and prognosis. Given a potential, predictive and therapeutic value of Circulating Endothelial Progenitor Cells (CEPCs) in breast cancer, level of CEPCs in breast cancer would correlate with extent of the disease. Our aim was to utilize a convenient and specific technology to detect CEPCs in breast cancer patients. Methodology flow cytometric detection of CD14, CD133, and Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) in the peripheral blood were performed for the study groups. Level of CEPCs was significantly higher in patients when compared with control. This was due to production of CEPCs by breast cancer tissue. Also level of CEPCs was significantly correlated with the size of the tumor and the stage of breast cancer. The level of CEPCs was significantly associated with the tumor metastasis site and size. There was highly statistically significant increase of CEPCs mean fluorescence intensity (Mx, My) with disease progression respectively as compared with controls. Since CEPCs may reflect the ability of a tumor to recruit the vascular infrastructure required to grow and metastasize, they may serve as a surrogate marker for disease recurrence and prognosis in a way similar to the evolution of use of tumor signatures. Since CEPCs may reflect the ability of a tumor to recruit the vascular infrastructure required to grow and metastasize, so the level of CEPCs can be used as a prognostic factor.

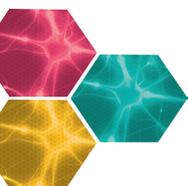


## **Remediation of Marine Sites from Crude Oil Contaminations Using Different Degradation Technology**

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Thirty nine bacteria were isolated from Ras-Gharieb at Red Sea, Egypt; ten of them (RG1-RG10), were selected according to their positive reaction with Tween-20 indicating their ability to produce lipase enzymes. RG1, RG4 and RG8 have the ability to produce highest lipase activity. Only RG1 contains a plasmid of a molecular weight 14 kb. The plasmid was transformed into *E. coli*  $\alpha$ -DH5 cells then tested on Tw-20, and showed their ability to degrade crude oil by forming a tar ball. RG1 strain was identified to the genus level as *Burkholderia pseudomallei*. PCR was carried out followed by 16S rRNA for confirming the biochemical identification. The parameters needed to maximize the degradation of crude oil as revealed in this study are pH 7.2, temperature 25°C, agitation 150 rpm and using marine medium. Therefore, bioremediation is being increasingly seen as an effective, environmentally benign treatment for shorelines contamination as a result of marine oil spills.



## **Suppressing HIF-1 $\alpha$ and c-Myc by Some Natural Extracts enhances Apoptosis and inhibits Glycolysis**

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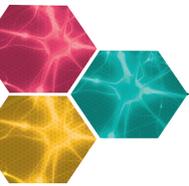
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Nearly seven decades after Warburg discovery, researchers have refocused again on targeting metabolic dependencies of malignant tumors as a novel approach in cancer care. Currently, efforts are exerted to introduce compounds that can target key regulators to correct altered cancer metabolism. Many natural products with anticancer activity in fact prevent deregulated cancer metabolism. HIF-1 $\alpha$  and c-Myc are known of their involvement in glycolysis and apoptosis regulation. This study was conducted to examine the effect of some natural extracts on the expression of HIF-1 $\alpha$  and c-Myc. It also aimed at determining some cellular proteins and enzymes involved in glycolysis [lactate dehydrogenase-A (LDH-A) and pyruvate kinase M2 (PK-M2)] as well as apoptosis [caspase-3 and -9] which are usually linked to HIF-1 $\alpha$  and c-Myc expression. Cytotoxic potential in terms of IC<sub>50</sub> was determined for avocado and ruta graveleones extracts in HepG2 cell line. HepG2 cells were then treated avocado (dose 40  $\mu$ g/ml) and ruta graveleones (dose 50  $\mu$ g/ml) extracts. HIF-1 $\alpha$  and c-Myc expression as well as LDH-A, PK-M2, caspase-3 and -9 were determined on protein level; by ELISA and mRNA level; by real time-PCR. A correlation between HIF-1 $\alpha$  and c-Myc on one hand and LDH-A, PK-M2, caspase-3 and -9 on the other hand was performed. The IC<sub>50</sub> determined after HepG2 cells treatment with avocado and ruta graveleones were 37.46  $\mu$ g/ml and 63.99  $\mu$ g/ml respectively. HIF-1 $\alpha$  and c-Myc expression as well as LDH-A and PK-M2 levels were significantly decreased. Caspase-3 and -9 levels were significantly increased. A strong positive correlation between HIF-1 $\alpha$  and c-Myc and LDH-A and PK-M2 was observed, while a strong negative correlation between HIF-1 $\alpha$  and c-Myc and Caspase-3 and -9 was obtained. Avocado and ruta graveleones extracts have a cytotoxic effect on HepG2 cells and when used in lower doses, they have an anti-glycolysis and pro-apoptotic effect.



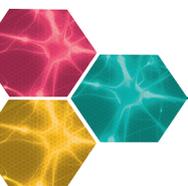
## **Evaluation of Fixed and Automatic Tube Current Modulation Based on Radiation Dose and Image Quality**

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The purpose of this study was to compare the radiation dose and image quality between Automatic Tube Current Modulation (ATCM) and Fixed Tube Current (FTC) techniques in computed tomography (CT) of multiphasic examination for the liver. The study included fifty cases examined by Multiphasic CT of the liver, the study was performed following scan steps according to hepatic circulatory phases namely arterial, portal, venous, and equilibrium phase. All phases are done using identical scan and reconstruction parameters except for the tube current. Portal phase was done using FTC technique while venous phase was performed using ATCM technique, the portal and venous phase were compared regarding their radiation dose values and image quality. Radiation dose measurements of the cases were generated automatically by the CT unit with a calculation of Dose Index Volume (CTDI<sub>vol</sub>). The image quality was quantitatively evaluated using Contrast-to-Noise Ratio (CNR). The results showed that the average of CTDI<sub>vol</sub> was found to be 26.07 mGy using FTC, and 13.71 mGy using ATCM techniques, there was a difference in CTDI<sub>vol</sub> between two techniques. Examination performed using FTC had an average value of CNR 28.93 HU while those using ATCM were 29.98 HU, there was no difference in CNR of the image between two techniques. In conclusion, by using ATCM, the radiation dose in multiphasic CT examinations of the liver can effectively be reduced with the maintenance of high image quality.



## **Studies on Recombinant Cellulase Cloned by PCR from *Bacillus Subtilis* 276NS**

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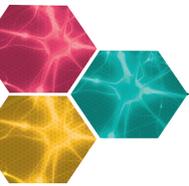
Fishing, cloning and expression for cellulase gene was applied in this study. The cloned cellulase gene of the experimental bacterium (276NS) was expressed into pCYTEXP1 under the regulated  $\lambda$  promoter as well as the cellulase. The gene was cloned directly by PCR then expressed into pCYTEXP1. The sequencing of 985bps and comparative similarity search of cell 276 of the experimental bacterium encodes a polypeptide of 328 amino acids residues using BLAST revealed 58% similarity to  $\beta$ -glucanase gene of *Bacillus subtilis* (ac: AAK94871). Multiple-alignment between cellulase of the experimental bacterium with the closest sequences showed that 199 amino acids are different among 328 amino acids residues of cellulase (cell276). Cell 276 gene was fished from genomic DNA of *Bacillus subtilis* and was cloned in *E. coli* DH5-alpha using plasmid pCYTEX. Heat shift induced the expression of cell276 enzyme. Clone harboring recombinant plasmid cell 276 were varified by a method induction using CMC plate and by PCR reaction using specific primer of cellulase and universal primer of vector, the cell276 gave  $\approx 63$  Kd active at 50°C and pH 8. The characterization and effect of different compounds (different cations, solvents and glycerol) on recombinant cell 276 enzyme activity were studied in this study.

## Histopathological and Biochemical Effects of the Thiocarbamate Herbicide, Thiobencarb on Nile Tilapi

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Thiobencarb, a thiocarbamate herbicide that contaminates the aquatic ecosystems, was investigated for its toxicity on marine organisms. The aim of this study was to determine the effects of thiobencarb on the Acetylcholinesterase (AChE) of Nile tilapia (*Oreochromis niloticus*) fingerlings after exposure to different concentrations of thiobencarb and determining the effects of four different diets on reducing the toxic effect of thiobencarb on *O. niloticus*. Different sublethal exposure of Nile tilapia to 0.625, 1.25, 2.5, 5 ppm of thiobencarb for 24, 48, 72, 96 hr resulted in the inhibition of brain AChE. Percentage of inhibition was increased as a function of concentration and exposure time. The most noticeable decline in AChE was observed after 48 hr at 1.25 ppm. All fingerlings of Nile tilapia showed behavioral effects after the exposure. The results are significant for reporting thiobencarb as a toxic herbicide to fish early life stages under sublethal exposure. In diet trial experiment (*O. niloticus*) fingerlings were acclimated to culture conditions and fed on different diets for 60 days then the pesticide was added and the feeding routine continued for another 45 days after the treatment. Biochemical and histopathological studies were conducted on the Nile tilapia fingerlings. The diets that affect the activation of AChE in Nile tilapia were that contained parsley, licorice seeds and charcoal as the main ingredients where diet containing ginger has no effect. The conclusion was that diets which contained parsley and licorice seeds could be used as an alternative diet in feeding *O. niloticus*, as they are rich with antioxidants that includes luteolin, flavonoid that eradicates free radicals in the body that cause oxidative stress in cells.

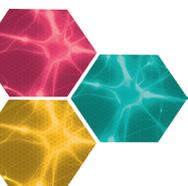


## **TFtoGI: Pipeline for Genome-Wide Identification of Genes Regulated by Certain Transcription Factor**

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The availability of genome and transcriptome data represent valuable resources for Gene Mining (GM). Meanwhile, there are a clear shortage in availability of powerful bioinformatic tools can assist to achieve this target at multi-genomes level. Therefore, the development of high throughput data analysis pipelines are strongly required. Identification of genes regulated by transcription factors within the completed genomes can provide an important resources for molecular breeding programs. We developed a fully automated Python pipeline called (TFtoGI) aims to genome-wide identification of genes regulated by certain Transcription Factor (TF). Starting with TFs complementary DNA (cDNA) sequences and multi-genomes of an organism as an input. TFtoGI can extract TFs sequences, find motifs, and search with them in promoter-specific genes of given organism. Finally, identified genes are classified into even genes with known functional proteins or genes with hypothetical proteins. We validated the efficiency and powerful of our developed pipeline using 469 TFs differentially expressed under drought stress in rice against 17 rice genomes. The results indicated the effectiveness and powerful ability of out developed pipeline in genome-wide identification of genes regulated by certain Transcription Factor (TF) in multi-genomes level.

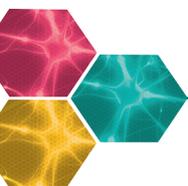


## The Polymorphisms of Vitamin D Receptor Gene Are Associated With Hashimoto's Thyroiditis among Egypt

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Hashimoto's thyroiditis is a common chronic autoimmune thyroid disease. Vitamin D is an important regulator of the (HT) immune system. It has been shown in various studies that vitamin D prevents the development of a myriad of autoimmune diseases. A plethora of studies in different populations have shown association between vitamin D receptor gene (VDR) polymorphisms and various autoimmune diseases, including (HT) however; results were conflicting. In this regard, this work was undertaken to assess the association of vitamin D status and VDR gene SNPs with (HT) susceptibility in a cohort of Egyptian (HT) patients. This study was performed on 59 patients (male/female: 8/51,  $33.44 \pm 12.02$  years) and 50 healthy matched controls (male/female: 10/40,  $34.28 \pm 15.21$  years). VDR gene SNPs {FokI (rs2228570), ApaI (rs7975232), BsmI (rs1544410) and TaqI (rs731236)} were genotyped by PCR-Restriction fragment length polymorphism. Serum levels of 25(OH)D<sub>3</sub> was performed using ELISA technique. Our results depicted that the prevalence of vitamin D deficiency among (HT) patients was significantly higher than in controls ( $P < 0.001$ ). Regarding FokI SNP, the genotype "FF" was more prevalent among controls than among patients (50.8% in patients vs. 76.0% in controls;  $P = 0.007$ ) while "Ff" genotype was dominant among (HT) patients (47.5% in patients vs. 24.0% in controls;  $P = 0.012$ ). Coming to BsmI SNP, the genotype "Bb" was more common among (HT) patients (59.3% in patients vs. 38.0% in controls;  $P = 0.027$ ). In contrast, "bb" genotype was common among controls (10.2% in patients vs. 30.0% in controls;  $P = 0.012$ ). Moreover, there were no statistically significant differences in the genotype frequencies of ApaI and TaqI polymorphisms among (HT) patients and controls. Our results clearly demonstrate that vitamin D deficiency, the "Ff" genotype of FokI SNP and "Bb" genotype of BsmI SNP are strongly associated with increased risk to (HT) disease in our cohort of Egyptian (HT) patients.

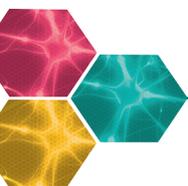


## **Preparation and Application of Chitosan-Metal Oxide Nanoparticles as Adsorbents for Pesticides Removal**

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The frequent detection of pesticides in water sources is of great concern to the public, to authorities and all those involved in water production, wastewater treatment and reuse. In addition, potentially adverse on health effects associated with such pollutants, even at very small concentrations. There can be no doubt that the Solid Phase Extraction (SPE) is today the most popular sample extraction technique. The current study aims to prepare two types of chitosan-metal oxide nanoparticles including Chitosan-Copper Oxide (Ch-Cu) and Chitosan-Zinc oxide (Ch-Zn) using sol-gel precipitation mechanism for use as adsorbent materials for pesticides removal from wastewater. The design of core shell was implemented by metal oxide core with chitosan hard shell using crosslinking agents (glutaraldehyde and epichlorohydrin). In addition, the characterization of synthesized nanoparticles were investigated using Fourier Transform Infrared Spectrometry (FTIR), zeta potential and Scanning Electron Microscope (SEM). The FTIR confirmed the reaction of chitosan-metal oxide and crosslinking. The SEM explained that the nanoparticles have spherical morphology and nano-size, where the Ch-Cu have an average size of 93.74 nm while the Ch-Zn have an average size 97.95 nm. The surface charge of both prepared nanoparticles had a positive charge. Plackett-Burman experimental design was applied to study the adsorption kinetics and the effects of pH, concentration of pesticides, agitation time and temperature on the adsorption and removal of pesticides from water samples. Moreover, the SPE cartridges packed with synthesized nanoparticles were applied in removal of some pesticides including abamectin, diazinon, fenamiphos, imidacloprid, lambda-cyhalothrin, methomyl and thiophanate-methyl from water samples. The results of kinetics study indicated that the optimum conditions of adsorption and removal were pH 7, 25°C and 25 minutes agitation time. The removal efficiency of Ch-Zn was higher than Ch-Cu, but both of them have a high removal efficiency up to 99% for the investigated pesticides.



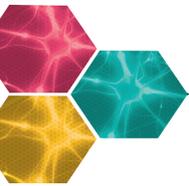
## **A Pilot Study on Stat3 Gene Polymorphisms Reveals Association with Hashimoto's Thyroiditis among Egypt**

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Hashimoto Thyroiditis (HT) is a common autoimmune disease. It is thought to arise from the interactions between genetic and epigenetic factors and various environmental triggers. Janus Kinase (JAK)–Signal Transducer and Activator of Transcription (STAT) pathway is as an evolutionarily conserved signaling pathway employed by diverse cytokines, interferons, growth factors, and other related molecules. Although STAT3 gene polymorphisms have been studied in various autoimmune diseases including (HT), but there were discrepancies in the literature regarding the association between STAT3 SNPs and (HT). In this context, the present case control study is an attempt to investigate the association between HT disease susceptibility and STAT3 gene SNPs namely {(rs744166), (rs2293152) and (rs3809758)}. To the best of our knowledge, the present work is the first to address such association among Egyptian (HT) patients. This work was conducted on 59 patients and 50 matched controls. STAT3 gene SNPs were genotyped by PCR-Restriction fragment length polymorphism. Our results inferred that the GG genotype of rs2293152 may reduce individual's (HT) susceptibility being less in (HT) patients than controls ( $P = 0.014$ ,  $OR = 0.359$ ,  $95\% CI = 0.156 - 0.825$ ). On the contrary, the GC genotype was highly associated with HT as it was more predominant in patients than in controls ( $P = 0.004$ ,  $OR = 3.145$ ), ( $95\% CI = 1.429 - 6.923$ ). Regarding rs3809758 SNP, all patients were carrying AG genotype suggesting a strong association with (HT) as compared to controls ( $P < 0.001$ ). On the other hand, AA genotype was significantly more frequent in controls than (HT) patients ( $P < 0.001$ ). Considering rs744166 polymorphism, there were no statistically significant differences in the genotype and allele frequencies between (HT) patients and controls. To sum up, the current findings strongly highlight the association between STAT3 gene polymorphisms and (HT) disease thus adding STAT3 gene to the list of (HT) predisposing genes.

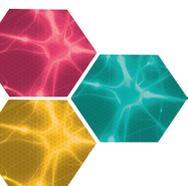


## Isolation of Glyoxalase II (gly II) and Salt Overly Sensitive (SOS2) Alleles from Egyptian Sorghum

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Two salt stress-related alleles namely, glyoxalase II (gly II) and Salt Overly Sensitive (SOS2) were isolated by RT-PCR from Egyptian sorghum cv. R3. The sequencing results confirmed the isolation of Sbgly II and SbSOS2 alleles, respectively. Both alleles were cloned separately into the pYES2 expression vector for expression in *Saccharomyces cerevisiae*. Yeast growth under different NaCl stress concentration, i.e. 0, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2 and 2.3 M. The results revealed significant growth differences between the transgenic yeast and control. Proline accumulation increased significantly in all transgenic yeasts with gly II and SOS2. While, these values were significantly decreased in the controls by increasing NaCl concentration. The present results suggested that the Sbgly II and SbSOS2 could be mediated improving the salt tolerance of different eukaryotic systems.

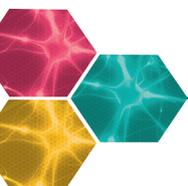


## Click Chemistry Established DNA Aptamer-based Fluorescence Assay of Tetracycline

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Tetracycline is an antibiotic which has been excessively used in human and animal livestock. Hence, several reports indicated its presence in residual amounts in various food and environmental samples. Given the need for selective, simple and accurate detection in biological, food and environmental samples, we proposed a new sensitive methodology for tetracycline assay that depends on complementation of DNA aptamer splits. CuS nanoparticles are prepared so that they contain carboxylic groups, which enabled coupling with the first DNA split via Carbodiimide chemistry. The other DNA split was decorated with Biotin, which enabled its coupling with streptavidin MagneSphere Paramagnetic Particles (PMPs). Complementation of the two-aptamer splits happened only in the presence of tetracycline and the subsequent sandwich is separated via a magnet. After the release of Cu(II), it was reduced to Cu(I) by sodium ascorbate, and finally catalyzed the click reaction between fluorogenic 3-azido-7-hydroxycoumarin and propargyl alcohol to afford the corresponding fluorescent 1, 4-disubstituted-1, 2, 3-triazole. The fluorescence signal produced was dependent on tetracycline concentration. Optimization of experimental conditions, validation, specificity and applicability of the method were studied and demonstrated.



## **Stability-Indicating HPLC-DAD Determination of Amprolium HCl and Ethopabate in Veterinary Powder**

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This work deals with the development and validation of a stability-indicating High Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) method for simultaneous determination of Amprolium HCl (APH) and Ethopabate (EPB). To the best of our knowledge, no stability indicating method has been reported for analysis of this binary mixture. Effective chromatographic separation was achieved using Kromasil CN column (4.6 × 250 mm) with gradient elution of the mobile phase composed of sodium hexane sulfonate and methanol. The quantification of ABH and EPB was based on measuring their peak areas at 266 nm. APH and EPB peaks eluted at retention times of 10.42 and 18.53 min, respectively. Analytical performance of the proposed HPLC procedure was thoroughly validated with respect to system suitability, linearity, ranges, precision, accuracy, specificity, robustness, detection and quantification limits. The linearity ranges for ABH and EPB were 1.5–240 and 1–160 µg/mL, respectively, with correlation coefficients > 0.9999. The analytes were subjected to forced-degradation conditions of neutral, acidic and alkaline hydrolysis, oxidation and thermal degradation. The proposed method proved to be stability-indicating by resolution of the analytes from their forced-degradation products. Moreover, the specificity of the method was verified by resolution of the analytes from about 22 pharmaceutical compounds commonly used in antimicrobial veterinary products. The validated HPLC method was successfully applied to the analysis of the cited compounds in their combined veterinary powder dosage form, in addition it was implemented in the accelerated stability study of the dosage form when stored for 6 months at 40°C and 75% RH. The proposed method made use of DAD as a tool for peak identity and purity confirmation.



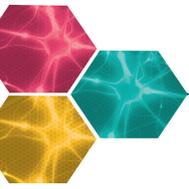
## ***In-Vitro* Evaluation of the Potential Antitumor Effect of GANT61 and BI-847325 in the Treatment of Lung Cancer**

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Despite the huge efforts employed to implement novel chemotherapeutic paradigms for the treatment of lung cancer, the disease still remains one of the major concerns worldwide. Targeting molecular pathways such as Hedgehog and MAPK pathways is a new hope in the treatment of lung cancer. In this context, the work was undertaken to evaluate the antitumor effects of GANT61 (an inhibitor of downstream targets of Hedgehog pathway), BI-847325 (a dual MEK and Aurora kinase inhibitor) and their combination on A549 adenocarcinoma lung cancer cell line. A549 cells were maintained as a monolayer culture in Dulbecco's Modified Eagle's Media. Cells were then passaged when they were 80% confluent. Cell proliferation assay was performed to determine the IC<sub>50</sub> of both drugs. A549 cells were treated with GANT61, BI-847325 and their combination for 3 consecutive days. Cells were collected and pellets were stored at -80°C. The protein levels of Caspase3, Bax, Mcl-1, CyclinD1, VEGF, ERK 1/2, p-AKT and PhH3 were measured using ELISA technique. GLI1 gene expression was assessed by quantitative real time PCR. Our data inferred that the IC<sub>50</sub> of GANT61 and BI-847325 were 4.16 μM and 28.63 μM respectively. On one hand, our results demonstrated that Caspase3 and Bax protein levels were elevated. On the other hand, Mcl-1, CyclinD1, VEGF, ERK 1/2, p-AKT and PhH3 levels were significantly reduced by both drugs and their combination relative to the control group. GLI1 gene expression level was down-regulated. To sum, both drugs and their combinations were able to inhibit cell growth and survival but activate the apoptotic pathway. Both drugs conferred a profound negative impact on the crosstalk between MAPK and PI3K/AKT/mTOR pathways, as well as hedgehog and PI3K/AKT/mTOR pathways. Further *in vitro* and *in vivo* studies are needed to support our promising results.

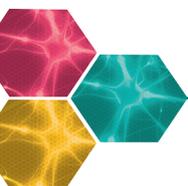


## **C9orf72 Expansion Disrupts ATM-mediated Chromosomal Break Repair**

Callum Walker, Saul Herranz-Martin, Evangelia Karyka, Chunyan Liao, Katherine Lewi, Waheba Elsayed, Vera Lukashchuk, Shih-Chieh Chiang, Swagat Ray, Pdraig J. Mulcahy, Mateusz Jurga, Ioannis Tsagakis, Tommaso Iannitti, Jayanth Chandran, Ian Coldicott, Kurt J. De Vos, Mohamed K. Hassan, Adrian Higginbottom, Pamela J. Shaw, Guillaume M. Hautbergue, Mimoun Azzouz, Sherif F. El-Khamisy

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Hexanucleotide repeat expansions represent the most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and frontotemporal dementia, though the mechanisms by which such expansions cause neurodegeneration are poorly understood. We report elevated levels of DNA–RNA hybrids (R-loops) and double strand breaks in rat neurons, human cells and C9orf72 ALS patient spinal cord tissues. Accumulation of endogenous DNA damage is concomitant with defective ATM-mediated DNA repair signaling and accumulation of protein-linked DNA breaks. We reveal that defective ATM-mediated DNA repair is a consequence of P62 accumulation, which impairs H2A ubiquitylation and perturbs ATM signaling. Virus-mediated expression of C9orf72-related RNA and dipeptide repeats in the mouse central nervous system increases double strand breaks and ATM defects and triggers neurodegeneration. These findings identify R-loops, double strand breaks and defective ATM-mediated repair as pathological consequences of C9orf72 expansions and suggest that C9orf72-linked neurodegeneration is driven at least partly by genomic instability.



## Stability Indicating HPTLC Method for the Simultaneous Determination of Biotin and Thioctic Acid

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A rapid, sensitive, selective and stability indicating high performance thin layer chromatographic method was developed and validated for the simultaneous estimation of Biotin (BO) and Thioctic acid (TH) in their pharmaceutical dosage form. The method employed HPTLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of chloroform:methanol:ammonia (6:4:0.01 v/v/v). This system was found to give compact bands for both BO (Rf value of  $0.25 \pm 0.02$ ) and TH (Rf value of  $0.65 \pm 0.02$ ). Spectrodensitometric scanning was performed at a wavelength of 215 nm. Regression data for the calibration plots showed good linear relationship with r values not less than 0.999 and concentration ranges of 5–20  $\mu\text{g}/\text{band}$  for BO and 5–30  $\mu\text{g}/\text{band}$  for TH. The method was validated for precision, accuracy and ruggedness. Both drugs do not undergo degradation under thermal conditions but showed significant degradation under acidic and basic conditions. Biotin samples degraded with HCl showed additional peak at Rf value of 0.86 and that of thioctic acid degraded with NaOH showed additional peak at Rf value 0.53. Statistical analysis proves that the method is reproducible and selective for the simultaneous estimation of BO and TH. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating one.

## Cardiac Protective Effects of the Angiotensin Receptor Blockers Valsartan and Losartan in Type 1 Diabetic Rats

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Nabil Abdel Ghany, Nermeen Shaffie, Somaia Ahmed Nada,  
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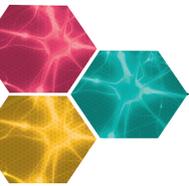
Augmentation of Angiotensin II (Ang II) signaling with the subsequent overproduction of the Reactive Oxygen Species (ROS) has been documented to be involved in the pathogenesis of the diabetes-induced cardiovascular complications.

*Objective:* This study aimed to assess the cardio-protective effects of long term treatment with the Angiotensin II type 1 (AT1) receptors blockers, valsartan or losartan, on the cardiovascular complications associated with diabetes in comparison with the classical antidiabetic drug, metformin. We have also evaluated the changes in cardiac, pancreatic, hepatic and renal tissue levels of chosen trace elements which could affect oxidative status.

Animals were randomly assigned into one normal group, one group of untreated Streptozotocin (STZ) (45 mg/kg)-induced diabetic rats, and five groups of STZ diabetic rats that received different daily oral treatments over a six week period as follows: metformin (100 mg/kg), valsartan (7 and 14 mg/kg), and losartan (5 and 10 mg/kg). At the end of the study ECG and heart rate variability were assessed. Blood samples were withdrawn for the estimation of serum cardiac troponin T and serum Angiotensin (1-7). Animals were sacrificed, and the heart, pancreas, liver and kidney were dissected, the heart was weighed for heart weight index calculation, and then carefully sectioned into two parts, one part for the histopathological examination while the other part along with other harvested organs were utilized for the trace elements evaluations.

The STZ-induced diabetic rats exhibited significant ECG changes, increased sympathetic activity, increased heart weight index, disturbances in the trace element content, as well as cardiac histopathological aberration compared with normal rats. No significant changes were observed on the serum troponin T and Ang (1-7) concentrations. Treatment with metformin, valsartan and losartan significantly ameliorated the disrupted cardiac functional and structural aberrations induced by the long term diabetes. In addition, the treatment with valsartan or losartan significantly increased the Ang (1-7) concentration with no effect on the cardiac troponin T.

In STZ diabetic rats, our data suggest that the beneficial effects afforded by valsartan and losartan treatments could be in part mediated through the increased synthesis of the cardioprotective Ang (1-7), and could also involve their effects on the tissues trace elements contents.

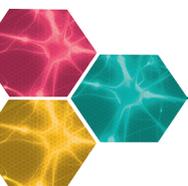


## **A Genetic Approach Using Yeast to Identify Novel Gene Functions for Genomic Maintenance Pathways**

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The DNA is the blueprint for life, as the valuable information carried by its nucleotides decides for the fate of organisms. When cells of living organisms encounter DNA damage, it should be rapidly repaired otherwise the organism will face negative consequences. Therefore, a various range of repair mechanisms co-exist in the cell working hand in hand to fix any damage the cell encounters. Systemic Lupus Erythematosus (SLE) is a very wide spread autoimmune disease, mostly affecting women. According to the Centre for Arab Genomic Studies, the incidence per 100,000 live births is 11–50. A subset of the SLE patients show mutations in RNase H2, an enzyme involved in DNA repair. In this project, we aim to identify new SLE disease-causing mutations. We performed a cDNA library screen in yeast to identify new players in genome maintenance pathways. The screen yielded 25 candidates, which were identified via sequencing. All candidates were re-tested on a small scale and 11 candidates were validated. We are currently studying the molecular mechanism through which the novel candidates achieve DNA repair in human cell lines. In parallel, we are analyzing SLE patients' samples to check for mutations in the candidate genes. Currently, the diagnosis of SLE in Egypt and all over the globe includes checking symptoms, Anti-Nuclear Antibody test, chest X-ray, checking serum creatinine and urinalysis. However, no genetic analysis and sequencing are done to determine the exact gene-causing mutation. On one hand, this is problematic because two patients can suffer from SLE but the cause for the disease in both patients is different on the genetic level. Thus, the medications that one patient responds for, would not affect the other. Identifying the disease causing mutations will allow faster disease diagnosis and open doors for development of personalized medicine tailored for patients suffering from specific mutations.



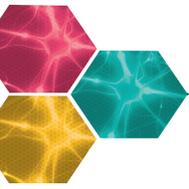
## **Modulation of L-dopa and Rasagiline Neuroprotection by Curcumin in Rotenone Model of Parkinson's Disease**

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Omar M.E. Abdel-Salam, Nermeen Shafee

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We investigated the effect of curcumin on brain oxidative stress, DNA fragmentation and motor changes in the rotenone-induced Parkinson's disease in mice. The possible modulation of the action of the anti-parkinsonian drugs L-dopa and rasigiline by curcumin was also studied. Mice were treated with rotenone 1.5 mg/kg subcutaneously every other day for two weeks. Starting from the first day of rotenone injection, mice were also treated with either curcumin (200 mg/kg), L-dopa (25 mg/kg), rasigiline (1 mg/kg), L-dopa + curcumin or rasigiline + curcumin. Results indicated that rotenone caused significant increase in brain lipid peroxidation (Malondialdehyde: MDA), and nitric oxide contents accompanied by depletion of reduced glutathione. Severe DNA fragmentation was seen in the striatum. Rotenone caused significant decrease in motor power in the wire hanging test and significantly impaired behaviors in the stair and wooden bar tests. In rotenone-treated mice, lipid peroxidation decreased by L-dopa and rasigiline while nitric oxide decreased by L-dopa + curcumin. Reduced glutathione increased by curcumin, L-dopa, rasigiline, L-dopa + curcumin or rasigiline + curcumin. These treatments also prevented DNA fragmentation and markedly improved the motor changes caused by rotenone in the stair and wooden bar tests. Motor power in the wire hanging test increased by curcumin and to lesser degree by L-dopa and rasigiline. Curcumin had an additive effect to L-dopa or rasigiline.

*Conclusions:* These data indicate that curcumin could be absorbed and reach the brain at concentrations sufficient to exert biochemical changes and to prevent DNA fragmentation caused by rotenone. Curcumin improved the motor power in mice with experimental Parkinson's disease. Curcumin showed some additive effects to L-dopa or rasigiline. Curcumin thus might be useful as an adjunct treatment in Parkinson's disease.

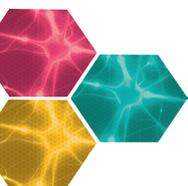


## Measuring Radon and Its Daughters in Makkah Tunnels Using Can Technique

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A new passive radon and its daughters detector based on a cylindrical metal can of radius 51 mm and height 102 mm with a hemispherical closed top with the radius of 51 mm was constructed. Such device has been proposed earlier by Somogyi, the device houses three detectors of CR-39, one exposed to the ambient air and two in different positions inside the cylinder. The detectors used to detect radon and its daughters. The detectors allow Rn-222, Po-218 and Po-214 concentrations to be determined separately. This device was applied to measure the radon and its daughters in Makkah Tunnels and the holy city of Makkah. The measurements show that the level of radon activity concentrations in Makkah Tunnels lies between 26-67 Bq/m<sup>3</sup>. In this work the radon activity concentrations have been measured for Makkah tunnels. The results show that the highest value of radon activity concentration (67 Bq/m<sup>3</sup>) is far below Radon active level (200-600 Bqm-3). This low level could be attributed to the good design of ventilation system in these Tunnels. However, routine measurement tasks should be arranged on a regular basis to monitor Radon activity concentrations.



## Synthesis, Anti-inflammatory Activity and Cyclooxygenase Inhibition of Pyrazole-Thiophenes Hybrids

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Treatment of inflammatory disorders is achieved by non-steroidal anti-inflammatory drugs that have been associated with gastrointestinal mucosal damage, ulceration and bleeding upon long term treatment. These side effects were found to be due to high Cyclooxygenase-1 (COX-1) versus Cyclooxygenase-2 (COX-2) selectivity. Hence, development of new anti-inflammatory agents that acts through COX-2 selective inhibition is highly recommended. Thiophenes, thienopyrimidines, thienotriazolo-pyrimidines and pyrazoles have attracted great interest due to their promising anti-inflammatory activities. Celecoxib is the most famous pyrazole-based, still marketed, COX-2 selective inhibitor. This research aims to synthesize new structure hybrids comprising basically pyrazole moiety attached to thiophene, thienopyrimidine or thienotriazolopyrimidine ring systems through various linkages aiming to develop promising anti-inflammatory agents that could act via selective COX-2 inhibition.

*Methodology:* Synthesis and screening of the anti-inflammatory activity of the three new groups of the target compounds were described. The anti-inflammatory activities were screened using rat-paw edema bioassay (acute and sub-acute inflammatory models) and using diclofenac sodium as a reference standard. The ulcerogenic, acute toxicity and inhibitory activities of COX-1 and COX-2 of the most active compounds were also evaluated.

*Results:* Compounds 3, 6a, 9 and 11 displayed distinctive anti-inflammatory activities with a fast onset of action. Compounds 9 and 11 revealed superior gastrointestinal safety profile with high safety margin. The thienopyrimidine/pyrazole hybrid 9 was more potent than diclofenac sodium showing  $ED_{50} = 12.12$  mg/kg. It also demonstrated some selectivity towards COX-2 than COX-1 which may account for its low ulcerogenic potential. Docking of compound 9 into the active site of COX-2 revealed that it was strongly held through pyrimidine N1, amino and cyano functionalities. Therefore, thienopyrimidine/pyrazole hybrid could represent new gateway for the development of new anti-inflammatory agents with selective COX-2 inhibition activity and improved safety margins.

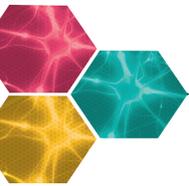


## **Biosynthesis, Characterization and Application of Metal-nanoparticles Produced by Achromobacter species**

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The development of environmentally benign procedures for the synthesis of metallic Nanoparticles (NPs) is a vital aspect in bionanotechnology applications in health care and environment. This study describes the simultaneous biosynthesis of Ag, Co, Cu, Fe and Zn NPs by employing *Achromobacter* sp. strain MMT, which was not reported previously. The physicochemical characterization confirmed the formation of quasi-spherical (22.1 nm), stable, monodisperse NPs with -52.5 mV of Ag (35%), Co (3.7%), Cu (5.8%), Fe (32%) and Zn (6.2%) concurrently, as indicated by TEM,  $\xi$  potential and EDX. The antimicrobial activity of mixed-NPs was assessed against Gram-negative, Gram-positive bacteria (aerobic and anaerobic), yeast and mold via well diffusion method. Besides, 50  $\mu\text{g/ml}$  of mixed-NPs inhibited 92%, 95% and 98% of *P. aeruginosa*, *S. aureus* biofilms and *C. vulgaris* growth, respectively. Moreover, morphological deformities caused by NPs were observed in the cells of *E. coli*, *S. aureus*, *C. perfringens*, *C. albicans* and *C. vulgaris* as elucidated by electron microscopy (Transmission and Scanning). Interestingly, the mixed-NPs exhibited promise synergistic biocide efficiency against wide spectrum of microorganisms which encourage their applications in adjuvant therapy and water/wastewater purification for controlling multiple drug resistant microorganisms.

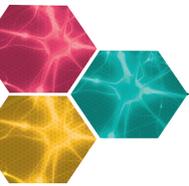


## Future Prospects for Conservation of the Endangered Species *Ginkgo biloba L.*

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*Ginkgo biloba L.* is the most ancient living gymnosperm and is a precious medicinal plant endemic in China. Although cultivated trees exist throughout the world, there is uncertainty regarding the future of *G. biloba* in the wild, which is currently confined to Xitianmu Mt., in China. IUCN listed the species as “endangered” due to the rapid decline in the trees around the world, for which conservation measures are required. *Ex situ* conservation requires identifying areas in which viable populations can be maintained. The ambiguity about the status of this species stimulated the current study aiming at predicting the potential distribution of suitability areas for propagation of *G. biloba* using MaxEnt modelling approach. Bioclimatic data were used for building the habitat suitability prediction models. Models were calibrated using 70% of the initial data sets and evaluated on the remaining 30% using the area under ROC curve (AUC) measure. The results revealed that five of the 18 bioclimatic variables used to build the habitat suitability models (namely annual mean temperature, precipitation of driest quarter, mean temperature of coldest quarter, mean diurnal range and temperature seasonality) have highly contributed to the predictive power of the model; with collective contribution importance of 97.1% and permutation importance of 91.8%. The results showed that Maxent accurately predicted the species suitability areas with AUC=0.95. The study also analyzed the threats to the species from climate change through projecting the changes in the suitability areas based on downscaled climate scenarios from CMIP5 (IPPC 5th Assessment). Conservation action for *G. biloba* to prevent its extinction should focus on translocation of high-yielding individuals to the suitable areas using the appropriate propagation techniques. It is hoped that the outcomes from the current study would help in providing more protection to this species.

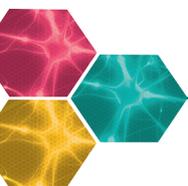


## **Bioinformatics Meta-analysis of Tumor Suppressor MicroRNA in Hepatic Cancer Stem Cells**

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Mahmoud El Hefnawi

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The Hepatocellular Carcinoma (HCC) is the third leading cause of death in cancer patients, due to the multi-drug resistance and poor prognosis. Hepatic Cancer Stem Cells (HCSCs) are considered as the main players for the tumor initiation, metastasis, drug resistance and recurrence. There is a growing evidence supporting miRNAs as key regulators for the HCSCs, but still more details are vague about how miRNAs modulate the HCSC mechanisms for HCC development. We are aiming here to present a panel of HCSCs specific miRNAs, their targets and pathways to be a step forward suggesting the selected candidates as tumor suppressor miRNAs. To achieve our goal, we collected 18 differentially down-regulated miRNAs and their experimentally validated and predicted up-regulated targets from various resources. We did extensive research through literature and employed bioinformatics tools, such as GEO database, miRbase, miRWalk, Cytoscape, DAVID bioinformatics resources, MirOb interactome and Reactome softwares. Then, we dissected all the resulted data for predicting new targets and constructing a comprehensive network of HCSC-specific miRNA-targets-pathways and biological processes. As a result, we found some hub miRNAs and new targets enriched for many cancer cell stemness hallmarks serving the pre-metastatic niche and chemoresistance nature of the CSC, which suggest these miRNAs role in the HCSCs neoplastic transformation. In addition, 2 miRNAs might have also oncogenic role, despite their low HCSC expression. Further, we provided new insights on the role of the selected miRNAs in controlling neurogenesis and metabolic activity that promote the stemness axis. Finally, we revealed the role of the selected miRNAs on orchestrating 4 HCSC pathways and especially on the TGF- $\beta$  signaling pathway, emphasizing its involvement in the development of HCSCs. In conclusion, our results strongly recommend the selected miRNA to be therapeutic agents against HCSCs transformation and the liver cancer development in the coming future.



## **Investigating Natural and Synthetic Curcumin Analogues for Nose-to-Brain Treatment of Alzheimer's Disease**

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This study introduces a new idea of utilising several biochemoinformatics tools in comparing two bio-similar natural phytochemicals viz. curcumin and Bisdemethoxycurcumin (BDMC) in order to select a potential nose-to-brain remedy for Alzheimer's disease. The comparison encompassed all levels starting from loading the drug in a certain nano-carrier; PLGA nanoparticles, to the biopharmaceutical level investigating the interaction with mucin and inhibition of P-gp blood–brain barrier efflux pumps. Finally, the therapeutic level was investigated by studying the interaction with pharmacological targets such as the amyloid peptide plaques and cyclooxygenase2 enzyme responsible for the inflammatory reactions of the studied disease. The comparison revealed the superiority of curcumin over BDMC. Five new analogues were also hypothesised where diethoxybisdemethoxycurcumin was recommended as a superior molecule. This work introduced the virtual utilisation of biochemoinformatics tools as a reliable and economic alternative to the exhausting and resources-consuming wet-lab experimentation.



## Effect of Mineral, Nano and Bio-fertilization on Nitrogen Content and Yield of *Salvia Officinalis*

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Nanotechnology and bio-fertilizers are represented as the most important tools in modern agriculture and anticipated to become a driving economic force in the near future. They focus on sustainability and protection of agriculturally produced foods, including crops for human consumption and animal feeding, provides new agrochemical agents and new delivery systems to improve crop productivity, and promises to reduce pesticide use. Nanotechnology is a novel discovery being explored in almost all the fields and is benefitted too; it may provide keener solutions for the current problems in the field of agriculture. A field experiment was carried out at Balaza Research Station of Desert Research Center, North Sinai (located at 29° 32'28" N and 32° 39'25" E), to study the effects of soil and foliar application for nitrogen fertilizer (urea), nanourea (nN) and biofertilization (*Azotobacter chroococcum*), on chemical composition and productivity of *Salvia officinalis* plant. Treatments were, two foliar applications of nN (250 and 500 ppm, urea (40 and 80KgN/faddan) and *A. chroococcum* soil and foliar application, using Split-plot design with eight treatments and three replications per each treatment, two Cuts, during 2017. Results showed that, foliar (nN 500 ppm) in two cuts increased plant height, Fresh and dry weight, whereas the lowest value were recorded in the control. The treatments Urea 80 kgN/fad increased percentage of oil and nutrient content in two cuts. Also, growth, yield, nutrient uptake parameters and oil percentage increased by soil inculcation of *A. chroococcum* compared to foliar application, treatment nN500ppm+ S. I. of *A. chroococcum* gave the highest value of plant height, fresh, dry weight, Dehydrogenase activity and *Azotobacter* densities in rhizosphere of *Salvia officinalis*, while the treatment U80kg/fad. + S. I. of *A. chroococcum* gave the highest percentage of oil.

## **Spoon: *In Vitro* and *In Silico* PCR Analysis Tool for Primer Selectivity, Fine Mapping and Gene Discovery**

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We developed C-PERL-based software for performing *in silico* PCR analysis on genomic sequences. Spoon software simulates PCR reaction by running an approximate-match searching analysis on a user-entered primer pairs against the provided sequences. Spoon reports amplimers close or adjoin genes/SNPs and that are shared between *in vitro* and *in silico* PCR results in order to select the most appropriate amplimers for gene discovery. Spoon could be used for comparing physical and genetic maps, studying the primer set genome coverage for PCR-walking and NGS sequencing filling gaps using Sanger Sequencing. It also reports chromosomal anchored markers, which could be used for linkage and association mapping. In addition to human-readable output files, Spoon creates Circos configurations illustrate different *in silico* results, which will give the user the ability to merge different bioinformatics tools results with/without slight reformatting procedures.

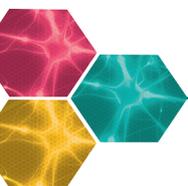


## **Development and Validation of Ternary H-Point and Artificial Neural Network Methods for Spectrophotometry**

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Two new, simple and specific analytical chemometric UV-spectrophotometric methods. First, an H-Point Standard Addition Method (HPSAM), and second a MATLAB-processed Artificial Neural Network (ANN) were developed and validated in accordance with International Conference on Harmonization (ICH) guidelines. Both methods were used for the simultaneous determination of ternary mixture of commonly co-administered cardiovascular drugs; Ticagrelor (TICA), Irbesartan (IRB) and Hydrochlorothiazide (HCT). For the first method, Ternary-HPSAM technique is based on the principle of dual wavelength spectrophotometry. For the determination of IRB in presence of TICA and HCT, the wavelength pair 214 nm and 228 nm has been chosen, where at 214 nm all three analytes exhibited the same absorbance value. Alternatively, for the determination of TICA and HCT, the wavelength pair 215 nm and 257 nm has been chosen. The wavelength choice was optimized according to the resolution of each analyte determination. Linear calibration curves were obtained over the concentration ranges of 10–30, 0.5–2 and 4–10 µg/mL for TICA, IRB and HCT respectively. For the second method: ANN (as a multivariate calibration method) was developed and applied for the simultaneous determination of three analytes. A training set of 90 different synthetic mixtures containing TICA, IRB and HCT, in non-linear wide concentration ranges between 0–30 µg/mL, 0–3 µg/mL and 0–10 µg/mL, respectively. Both methods were successfully applied on laboratory-made mixtures and spiked plasma samples. Satisfactory results of student's t- and F-variance ratio tests were obtained upon statistical comparison between the proposed methods.



## **Biological Synthesis and Characterization of Nano-adsorbent Magnetic iron for Phenol Decontamination**

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The biological synthesis of metal nanoparticles using microorganisms is more easy and ecofriendly than chemical and physical methods. Mainly Magnetic Iron Nanoparticles has special characters can be used to get rid of phenol. The purpose of this study was to consider the biological synthesis of magnetic iron nanoparticle and its ability in phenol decontamination. Iron nanoparticles were biosynthesized using *Metschnikowia pulcherrima* KM658981 under anaerobic growth condition. It was characterized by TEM, XRD, VSM, UV-visible. The evaluation of magnetic iron nanoparticles as nano-adsorbent was examined on phenol removal from aqueous solution. Different concentrations from iron nanoparticles (0.5–10 mg/100 ml) were added to aqueous solution of crystalline phenol (50 mg/l). The results revealed that the concentration 6 mg/100 ml was found to have the ability to dispose of 92% phenol contaminants from aqueous solution. Hence, the magnetic iron nanoparticles act as good phenol decontaminant in water and wastewater.

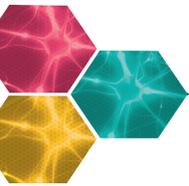


## **Biopolymer Extracted Ulvan from *Ulva Intestinalis Macroalgae* Towards Nanofibers Fabrication**

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Nanofibers fabricated from biocompatible polymers received a great attention in the new generation of biomedicine and nanotechnology. Hence, the following study aimed to extract biopolymer from green algae and fabricate nanofibrous blend that is suitable for biomedical applications. Ulvan was extracted from *Ulva intestinalis* with/without organic solvent pretreatment. For both resulted ulvan IR spectra, Molecular weight and chemical composition were determined. The measured parameters showed no significant differences between both samples. However, ulvan powder from pretreated sample showed pigment color contamination unlike non-pretreated one. Both extracted ulvan samples showed antibacterial activity against *Pseudomonas aeruginosa* but not against *Staphylococcus aureus*. Nanofibrous blend with Polyvinyl Alcohol (PVA) was fabricated using water as a solvent. It is noteworthy to mention that, non-pretreated samples showed beads free nanofibers, unlike the pretreated one. In conclusion, pretreatment using organic solvent is not needed in extracting ulvan as a biopolymer. In particular if it will be used in nanofibers fabrication especially for biomedical purpose.

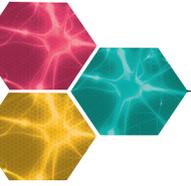


## Molecular Study on Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is the most common severe form of childhood muscular dystrophy affecting 1:5000 newborn males. The lack of functional dystrophin results in repeated cycles of muscle necrosis and regeneration leading to eventual replacement of muscle fibers by adipose and connective tissue. The aim of the study is to establish a baseline of the level of circulating serum miR-1, miR-206, miR-133, MM9-9, and TIMP-1 in a group of DMD naïve patients, and DMD patients who took the routine regimen. Fifteen blood samples were collected on EDTA, from 15 pediatric patients between the ages 5–13 years old, and were centrifuged; Eppendorf tubes of the supernatant were stored at –80 degrees, for further tests to be carried on them. Serum levels of Creatine Kinase, Lactate Dehydrogenase (LDH), Myoglobin, miR-1, miR-206, miR-133, MM9-9, and TIMP-1 were assayed and statistical tests were carried out, results showed significant difference of miR-1, miR-206, miR-133, MM9-9, and TIMP-1 concentration from the control, which implies that such biomarkers' concentration is reliable as a diagnostic tool for the early discovery of DMD.



## **DPMMD: A Database for Studying and Development Molecular Markers in Date Palm**

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In this work, we established the first Date Palm Molecular Markers Database (DPMMD). This database includes information of more than 3,611,400 DNA markers (such as, SSR, SSR-SNPs and SNP markers), genetic linkage maps, KEGG maps, DNA-barcode and all previously published date palm articles in PubMed since 1976 to 2017. Keyword searches for the markers, sequence data used for marker development and other information are also available through this database. DPMMD will be a useful tool for both basic and applied sciences, such as genomics, genetics, and molecular breeding in date palm.



## The Optimum Setting Temperature for Strawberry Freezing

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The purpose of this work was to determine the optimum air blast freezer setting temperature for freezing Festival strawberries grown in Egypt. The effect of sitting temperatures ( $-20^{\circ}\text{C}$ ,  $-25^{\circ}\text{C}$ , and  $-30^{\circ}\text{C}$ ) on the textural and technological properties was studied. Physical characteristics (weight, dimensions, and color) and biochemical analyses (moisture content, protein, fat, crude fiber, ash, and carbohydrates) of fresh fruits were determined. Three air blast freezer setting temperatures were used;  $-20^{\circ}\text{C}$ ,  $-25^{\circ}\text{C}$ , and  $-30^{\circ}\text{C}$  to reach a core temperature of  $-18^{\circ}\text{C}$  for the fruit, then fruits were thawed at room temperature until  $5^{\circ}\text{C}$ . The textural properties of the fruits were studied as affected by the Freezer Setting Temperature (FST) by applying the Texture Profile Analysis (TPA) test on the fresh fruits (control samples) and the thawed fruits (treated samples) using a texture analyzer. Also, the technological properties; color, Total Soluble Solids (TSS), Electrolyte Leakage (EL), Vitamin C (VC) and titratable acidity were studied as affected by FST. The Festival weight, length, width, and thickness were 17.92 g, 4.39 cm, 3.42 cm and 3.05 cm, respectively. Freezing and thawing curves (temperature vs. time) were plotted, and total freezing times were measured. The total freezing times at deferent setting temperatures ( $-20^{\circ}\text{C}$ ,  $-25^{\circ}\text{C}$ , and  $-30^{\circ}\text{C}$ ) for Festival (from  $10^{\circ}\text{C}$  initial temperature to  $-18^{\circ}\text{C}$  final temperature) were 72.83, 51.17, and 28.5 min, respectively; and the freezing rates were 0.376, 0.564, and  $0.994^{\circ}\text{C min}^{-1}$ , respectively. The TPA test illustrated that, the Hardness and Chewiness were significantly ( $p < 0.05$ ) affected by freezing/thawing process and FST. However, Cohesiveness and Springiness index were neither affected by freezing/thawing process nor FST. TSS, EL, VC and acidity were significantly affected by FST. The obtained results can be used to enhance stability, maintain quality, and extend shelf life of stored strawberry.



## **Development of AFLP, ISSR and RAPD Markers Related to High-Yield Component Traits in Jojoba**

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Jojoba is monogeneric dioecious shrub plant that is important to commerce as its seeds store liquid wax (40–60% by dry weight). Due to wide variations in yield, selection of female superior plants is the most important parameter for increasing yields in future populations. In this study, 50 female strains were evaluated for 13 traits representing morphology, seed characteristics and yield. Six jojoba strains representing the extremes for yield and seed weight traits were selected for molecular analysis. The selected strains were characterized using 8 AFLP, 16 ISSR and 30 RAPD primers/primer combinations. For yield, the AFLP, ISSR and RAPD produced 531, 138 and 325 total scorable bands with percentage polymorphism of 28.0, 35.5 and 35.6, respectively. While for seed weight, they generated 524, 135 and 317 total scorable bands with percentage polymorphism of 27.0, 31.1 and 34.0, respectively. A dendrogram based on UPGMA analysis of the used techniques and combined data were constructed for both yield and seed weight extremes. For yield, all dendrograms successfully grouped the superior strains in one cluster while with seed weight, the superior strains were grouped in one cluster except ISSR dendrograms. These results represent the first case study combining different molecular marker techniques, in addition to agronomical and morphological evaluation of 13 traits, in order to develop unique positive and negative markers that can be used to identify superior jojoba strains in early stages.



## HPLC-DAD Stability Study of Sacubitril/Valsartan (LCZ696) with Degradation Kinetics of Sacubitril

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A newly FDA approved therapy for treatment of heart failure {LCZ696, supramolecular complex of Sacubitril (SAC) and Valsartan (VAL), Entresto™} is analyzed with a stability indicating HPLC-DAD method. For the newly introduced SAC, there is little information about its stability under various stress conditions. The proposed chromatographic method was applied to the kinetic investigation of the acidic, alkaline and oxidative degradation of SAC with the estimation of its activation energy and half-life at room temperature by the aid of the Arrhenius plots. Kinetic investigation was conducted using either different strengths of HCl, NaOH and H<sub>2</sub>O<sub>2</sub> at one selected temperature or different temperature degrees, at one selected reagent strength, for the acidic, alkaline and oxidative stress conditions. The chromatographic method was achieved using Zorbax Eclipse plus-C18 (4.6 × 250 mm, 5 μm) with isocratic elution of mobile phase composed of acetonitrile and 0.025 M phosphate buffer (pH3) in a ratio of 60:40 (v/v). The mentioned drugs were resolved with retention times 4.5 and 5.6 min for VAL and SAC, respectively. Furthermore, both VAL and SAC were subjected to different forced degradation studies.

*Conclusion:* The proposed method could well resolve the parent drug peak from the degradation products peaks with peak identification and purity confirmation by using diode array detector. It was found that the pseudo first order kinetics was followed at each case for SAC degradation. Its half-lives at room temperature using 0.1M HCl, 0.01M NaOH and 15% H<sub>2</sub>O<sub>2</sub> were found to be 20.50, 2.76 and 51.58 hours.

## Maximization of Antimicrobial Metabolites of *Streptomyces Amritysarensis* EGANTI Using Plackett-Burman

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*Streptomyces amritysarensis* EGANTI was isolated from farming soil in the Menoufiya Governorate, Egypt. It showed broad-spectrum antimicrobial activity among thirty actinobacterial isolates which they were screened using agar-well diffusion method against (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus fumigatus* and *Candida albicans*). Its identification performed based on DNA barcoding using 16s rRNA gene and phylogenetic analysis. The gene sequence had been deposited in the GenBank Database at NCBI under accession number KY120367 using Prokaryotic 16S rRNA wizard tool. Yeast extract malt extract medium (ISP2) had been selected between five media (Starch-Nitrate medium, ISP4, ISP2, Medium D and Hobbs minimal medium) screened as it produced highest diameter for zone of inhibition against those microbial pathogens. We applied Plackett-Burman Design (PBD) for prediction of optimization range of the ten factors which led to maximization of antimicrobial metabolites against (*Staphylococcus aureus*, *Salmonella typhi* and *Aspergillus fumigatus*). The results of Pareto chart and main effect demonstrated that six factors ( $ZnSO_4 \cdot 7H_2O$ ,  $MgSO_4 \cdot 7H_2O$ ,  $FeSO_4 \cdot 7H_2O$ , malt extract, peptone and glucose) are significant and they all displayed positive effect except Malt extract was negative effect, which was obvious in the combination NO.7, the diameter reached 21.5 mm. Ultimately, PBD as statistical and mathematical approach is very crucial to save money, time and effort for Bioprocess optimization of antimicrobial metabolites.



## **Role of Mycorrhizae and Compost in Improving Nutrients Availability in Calcareous Soils**

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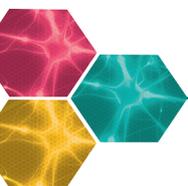
Calcareous soils cover more than 30% of the earth surface. These soils are suffering from macro- and micronutrients deficiency and have low content of organic matter. To find a solution, pots experiment was conducted to evaluate the role played by mycorrhizae and its product namely glomalin in improvement of some soil properties. Two soils were treated with mycorrhizae, compost and mycorrhizae combined with compost in comparison with controlled soils. Soil parameters like organic carbon and cation exchange capacity were significantly and positively correlated with glomalin production. As a result total nitrogen, available phosphorus, available potassium and soil micronutrients increased significantly among all treatments. On the other hand nitrogen, phosphorus, potassium, zinc and copper in maize plants were significantly increased among all treatments. In conclusion mycorrhizae and mycorrhizae combined with compost showed the highest positive effects on the studied soil properties.

## Cytotoxic T Lymphocytes Mediators And Serpinb9 in Coronary Atherosclerosis

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Cytotoxic T Lymphocytes (CTLs) play a pivotal role in atherosclerosis, the major cause of mortality in type 2 Diabetes Mellitus (DM). They mediate their activity mainly through proapoptotic protein Granzyme B (GZB) and Perforin (PRF). The imbalance between GZB/PRF system and proteinase inhibitor-9 (PI-9; SERPINB9), the only known inhibitor of human GZB has been demonstrated in atherosclerosis. However, the exact role of GZB/PRF system and PI-9 in atherosclerosis with the impact of type 2 DM and their contribution to hallmarks of atherosclerosis is not fully clear. Serum insulin, high sensitivity C-reactive protein (hs-CRP) and GZB levels were estimated by ELISA while mRNA expression levels of GZB, PRF and PI-9 in peripheral leucocytes and atherosclerotic tissues samples were quantified by TaqMan RT-PCR. *Results:* Serum insulin, hs-CRP and GZB levels were significantly higher in atherosclerotic patients compared to controls. There was a significant increase in GZB mRNA expression and significant reduction in PI-9 mRNA in both peripheral leucocytes and atherosclerotic lesions at  $P < 0.001$ ; on the other hand, PRF mRNA expression increased significantly only in atherosclerotic tissues at  $P < 0.001$ . With the impact of type 2 DM, only PI-9 mRNA showed more reduction in peripheral leucocytes and atherosclerotic tissues of diabetics than non-diabetics at  $P < 0.05$ . Moreover, regression analysis revealed that GZB and PI-9 were significant modulators for inflammation and insulin resistance. Interestingly, PI-9 mRNA expression in peripheral leucocytes was inversely contributed to CAD severity. *Conclusions:* GZB and PI-9 might be effective contributors for inflammation and insulin resistance in atherosclerosis. Circulating PI-9 mRNA might be a biomarker of CAD severity.



## **Combining Sulfasalazine with Imatinib Synergistically Inhibits Hepatocellular Carcinoma Cells**

Marium Muhamed Shamaa

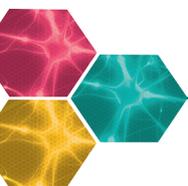
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Hepatocellular Carcinoma (HCC) is one of the lethal human malignancies. Lack of efficient therapy for advanced HCC is a pressing problem worldwide. Imatinib is a small molecule kinase inhibitor used to treat certain types of cancer. It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, but recent toxicity concerns suggest that new strategies for its use are needed. One strategy for reducing toxicity is to use lower doses of Imatinib in combination with other complementary mechanisms. NF $\kappa$ B is one of widely recognized positive regulator of cancer cell proliferation. It could be worthy to combine tyrosine kinase inhibitor with NF $\kappa$ B inhibitor for targeting of multiple signaling pathways involved in carcinogenesis. This study aimed to evaluate the potential anti-carcinogenic effects of either Imatinib and/or Sulfasalazine on HEPG2 cell line as a model of HCC.

One human HCC cell lines, HEPG2 was treated with Imatinib and Sulfasalazine, alone and in combination, and the effect of these treatments on growth, apoptosis, and expression of HIF-1 $\alpha$  was evaluated by ELISA and QRT-PCR, respectively.

Both drugs have modulated PI3K/AKT, BCR/ABL and NF $\kappa$ B pathways. On the molecular level, Imatinib and Sulfasalazine downregulated the gene expression level of HIF-1 $\alpha$ . When compared to the actions of either agent alone, the combination of low concentrations of Imatinib and Sulfasalazine resulted in enhanced inhibition of both cell growth and AKT activation, and increased the induction of apoptosis. Combination Index (CI) analysis showed that the growth inhibition effect was synergistic.

This study shows that Sulfasalazine synergistically potentiates the Imatinib-mediated antitumor effect. This finding establishes the foundation for clinical trials evaluating the efficacy of co-administration of Imatinib and Sulfasalazine as a treatment for HCC.



## Preparation and Characterizations of Chitosan-Essential Oil Nanoemulsions

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Naturally occurring polymers such as Chitosan is widely used in biomedical and pharmaceutical fields in various forms such as nanoparticles, capsules, and emulsions. This biopolymer has attractive applications in food technology, agriculture and drug delivery because of its biodegradability, biocompatibility, and nontoxic nature. The current study focuses on the use of nanotechnology-related food-preservation and nutrition for the preparation of Chitosan-essential oil nanoemulsions. Chitosan and Essential Oils (EOs) gained interests as natural antimicrobial agents for food preservation against foodborne pathogens and spoilage bacteria. EOs constituents characterized by low solubility in water, need to be incorporated in appropriate delivery systems to promote their efficiency. Three concentrations (0.5%, 1% and 3%) of four essential oils (*Mentha piperita*, *Punica granatum*, *Thymus vulgaris* and *Citrus limon*) in olive oil as a carrier at ratio of (1:4), were mixed with three concentrations of a biopolymer chitosan (0.5%, 1% and 2%) under continuous stirring for 30 minutes to prepare 12 different nanoemulsions. The characterizations of prepared nanoemulsions including viscosity, pH, thermodynamic stability, droplet size (nm) and Polydispersity Index (PDI) were investigated in details. The results showed that the nanoemulsions of *Punica granatum*, *Thymus vulgaris* and *Citrus limon* oils at 0.5:0.5:4%, Chitosan: EOs: carrier, respectively produced the lowest particles size (150.7, 99.2 and 169.5 nm, respectively) and with PDI values of 0.288, 0.242 and 0.310, respectively. However, the ratio of 1:1:4% of *Punica granatum* oil produced particles size of lower than 300 nm and PDI of 0.285, with good stability. The results suggest that the Chitosan-EOs nanoemulsions could be used in food preservation as antimicrobial agents and for shelf-life extension.

## Evaluation of The Potential Antitumor Effects of Gant61 and/or Dactolisib On Prostate Cancer Cells

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Prostate cancer comes in the fourth position and the second position among the most common cancers in both sexes and in men respectively worldwide. Aberrant activation of several signaling pathways is a major cause for disease progression to Castrate Resistant Prostate Cancer (CRPC). PI3K/Akt/mTOR and Hh/Gli pathways are major participants in progression to CRPC. The aim of this study is to assess the antitumor effects resulting from the targeting of the aforementioned pathways in PC3 cells. Drugs utilized were Dactolisib (dual PI3K/mTOR inhibitor) and GANT61 (GLI1 antagonist). Three replica of PC3 cells were assigned for each of the four treatment groups, vehicle control, Dactolisib-treated, GANT61-treated and Dactolisib/GANT61 combination treated groups. GLI1 gene expression was determined by qRT-PCR while p-Akt, pS6K1, cyclin D1, VEGFA, active caspase-3 and LC3 protein levels were determined by ELISA technique. Our results revealed that GLI1 gene expression was down-regulated as a result of Dactolisib, GANT61 and their combinations. In comparison to the control group, treatment of PC3 cells with Dactolisib and/or GANT61 have significantly reduced protein levels of p-Akt, S6K1, cyclin D1 and VEGFA. Dactolisib but not GANT61 treatment has resulted in high LC3 level while the addition of GANT61 to Dactolisib in a combination treatment has augmented its effect on LC3 level, On the other hand, only Dactolisib/GANT61 combination treatment has induced significant elevation in active caspase-3 level. In conclusion, Dactolisib/GANT61 combination was shown to be promising in PCA treatment which needs further *in vitro* and *in vivo* studies.



## Non-parametric Spectrophotometric Analysis of Olmesartan, Hydrochlorothiazide and Amlodipine

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Olmesartan Medoxomil (OLM), Hydrochlorothiazide (HCT) and Amlodipine besylate (AML) mixture is indicated for hypertension treatment. Few methods for their simultaneous analysis were reported. So, the aim of proposed work is to develop validated spectrophotometric techniques for their assay without prior separation. In addition, comparing the non-parametric linear regression method (Theil's method) in handling spectrophotometric data with the de facto least-squares regression method was achieved.

Absorption spectra of drugs and their mixtures were measured against methanol. Parameters as (derivative order, divisor concentration wavelength interval and wavelength) were optimized for the assay of each drug in presence of other.

### **(4DDRD) method for OLM**

The absorption spectra of OLM and its ternary mixture with HCT and AML were divided by the sum of the absorption spectra of HCT and AML as a (double divisor) and the ratio spectra were obtained. Fourth derivatives of the ratio spectra were plotted. The amount of OLM was determined by measuring the amplitude at 268 nm.

### **(4D) method for HCT**

The fourth derivative of the absorption spectra of HCT were measured at its  $\lambda_{\max} = 342$  nm. At this wavelength, no interference from OLM or AML occurred.

### **(0D) Direct spectrophotometric method for AML**

The zero order absorption spectra of AML were measured at its  $\lambda_{\max} = 360$  nm. No interference from OLM and HCT occurred at this wavelength.

Despite the complete spectral overlap of the drugs, the proposed spectrophotometric methods provide simple, accurate and reproducible assay of them without interference from each other or from excipients in tablets. The non-parametric method of regression enhances the linearity parameters especially upon the use of very small data sets frequently used in analytical work.