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CONTENTS

Preface

'Integrated Applied BioSciences' focus on the development of novel and science-driven solutions in all areas of biosciences including medicine, pharmaceutical sciences, agriculture, biotechnology, environment etc. Research and development in applied biosciences provide an impetus for new therapeutics and biopharmaceuticals to treat a wide-range of diseases across multiple therapeutic areas. Applied Biosciences has potential to enhance global food security, and enable groundbreaking research for greater sustainability, productivity, and nutritional density.

Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan organized International Conference on Applied Biosciences (ICAB2019) during December 5-6, 2019. Theme of the conference was **"*Reshaping Life Technologies*".** Hundreds of scientists, post-doctoral fellows and graduate students from all over Pakistan and other countries attended this conference. In this conference, an array of topics related to applied biosciences including Biochemistry, Bioinformatics, Metabolomics, Biotechnology, Cancer Biology, Halal Sciences, Genomics, Metagenomics, Proteomics, Health Biotechnology, Immunology, Infectious Diseases, Microbiology, Molecular Biology & Genetic Engineering, Molecular medicine, Neuro Sciences, Virology have been included.

In this issue of PJBMB, we are publishing abstracts of plenary, invited and session lectures delivered during the conference. Moreover, abstracts of young investigators presentations and poster presentations of this conference also included. Editorial board is grateful to the organizing committee of ICAB2019 for providing abstracts of oral and poster presentations for publication in PJBMB.

Prof. Dr. M. Kamran Azim

Executive Editor

Pakistan Journal of Biochemistry and Molecular Biology

ABSTRACTS OF
PLENARY LECTURES IN ICAB-2019

Understanding health and disease using Proteomics: Roadmap for future

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Proteomics deals with high throughput analysis of complete protein component of a cell, tissue or organ. Being indispensible molecule of living organisms, proteins are critical for maintaining homeostasis. Changes in protein expression levels are likely to affect function, hence, proteomics not only relates the genotype with phenotype; it also helps in translating genomic information into functional proteins. The techniques used in proteomics involve combination of wet lab and *in silico* methods. Two dimensional gel electrophoresis and Mass spectrometry have been method of choice from the beginning, but in recent years, the paradigm has shifted towards quantification and quantitative proteomics is gaining much popularity due to better accuracy and sensitivity. In recent years, Proteomics is being used to study tumor metastasis, biomarkers discovery, disease diagnosis and prognosis, drug discovery and protein-protein interaction to name a few. Our main interest has been on identification of therapeutic targets and diagnostic/ prognostic biomarkers. We have used proteomics approach to explore therapeutic potential of different drugs and their impact on signal transduction pathways in hepatocellular carcinoma and oral cancer cell lines. We have also identified and validated putative diagnostic/prognostic biomarkers from tissue and sera of cancer patients. Proteomics studies may, therefore, compliment genomics studies and can help us in understanding underlying molecular mechanisms involved in health and disease which can further help in developing therapeutic strategies.

Metagenomics for public health, biomedicine and environment

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Advent of high throughput DNA sequencing technologies has shifted the metagenomic research to characterize not only the composition but also the functional capabilities of microbial communities in diverse environments. Shotgun metagenomics is a robust technique that provides untargeted genetic information of all the microbial communities present in the sample. It also shows dominance over the marker based metagenomics which only targets a selected region instead of whole genomic content of organisms and might lead to arbitrary results. Metagenomics has proven to be a cost and time effective identification approach that employs the fusion of total genomic DNA isolation from a mixture of species and next generation sequencing. On the other hand, 16S rRNA based metagenomics is a distinctly feasible, rapid and cost-effective medium for the characterization of bacteria. This method has been made viable through merging of polymerase chain reaction (PCR) with high throughput next generation sequencing. During this presentation, application of metagenomics for advancement in public health, medicine and environment will be discussed.

Declining Taxonomic Expertise – A Way forward for Pakistan

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For centuries, people have been depending on the science of classical taxonomy for defining and naming groups of biological organisms on the basis of shared characteristics. The discipline is pivotal to identifying and enumerating the components of biological diversity which resultantly provides the foundation for future biodiversity conservation endeavors. The taxonomic expertise is, however, on a decline globally. Despite taxonomic research of the past 250 years, we have only been able to discover 14% of the land and 9% of the marine species. Keeping in view the time and cost required to undertaking giant task of discovery and documentation of all forms of life on earth, an affordable and rapid alternative is the need of the hour. DNA Barcoding offers an alternative to speed up this process and augment the science of taxonomy. A DNA barcode is a universally accepted short DNA sequence which can serve as diagnostic features for prompt and unambiguous identification of species. By using automated and standardized procedures and techniques, it aims to augment conventional methods of species discrimination. Besides, DNA barcoding brings both accelerated species diagnostics with a much larger accessibility of data. This molecular technique, unlike traditional taxonomic way of identification, does not need the whole organism or a particular adult stage of development for detection. In context of the quarantine, this is an overwhelming advantage.

Pakistan was among 150 countries that signed the Convention on Biological Diversity (CBD) at the 1992 Rio Earth Summit, and ratified it in 1994. As a matter of fact Pakistan's biodiversity is largely unknown and without detailed insight in perspective of changing climate and other factors. Besides, a little or least is known or characterized at genetic/molecular level of the biodiversity of Pakistan. With the immense advent of molecular biology techniques has enabled quick and reliable identification of species, their role in ecosystem and thereby their sustainable use.

To fill this gap and to cope with the mentioned scenario, Pakistan Barcode of Life (PakBOL), a network of academia, research organizations and private sector organizations committed to document and understand the country's biodiversity by employing the DNA-based identification systems is envisaged. With the launch of Pak BOL, Pakistan's DNA barcode research community has become part of the International Barcode of Life Consortium (iBOL), a long-established international network of organizations working to improve understanding of planetary biodiversity. Although Pakistan has been an informal participant of iBOL since 2010, Primarily the PakBOL initiative is focusing to inventory all species of the country in a swift, credible and cost effective manner. The species' inventory could be helpful in developing bio monitoring systems for invasive species and to track shifts in distributions and abundances of different species particularly from view point of Climate Change. The barcode system, on the other hand, can facilitate the quarantine and other concerned departments to curb illegal trade of precious animals, animal parts, medicinal plants etc. Besides, food authorities of different provinces can make use of DNA Barcoding to ascertain originality of various food items.

Next generation sequencing can guide personalized treatment for drug resistant Tuberculosis

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Multi-drug resistance (MDR) defined by MTB resistant to rifampicin (RIF) and isoniazid (INH) account for 4.2% of new TB cases and up to 16% of re-treatment and relapse TB cases in Pakistan. This translates to an estimated 15,000 MDR-TB cases. However, there were MDR/RR-TB: 3 475 laboratory confirmed cases of which approximately 30,000 were put on treatment. This suggests 12,000 missing MDR-TB cases. Further, resistance other than to RIF may be mistakenly treated as susceptible TB, increasing the chance of treatment failure, increasing disease burden, perpetuating morbidity and the period of transmission.

For MDR-TB treatment, new drugs have been recommended. However, rapid genotypic testing is not available for a number of these such as, Bedaquiline (BDQ), Linozelid (LZ), Clofazamin (Cz), Cycloserine (Cs), Pyrazinamide (PZA) or Ethionamide (Eth). Sequencing of MTB strains can provide reliable genotypic resistance assessment of strains. Initiating patients on standard treatments (TB and MDR-TB) without a comprehensive assessment of resistance to all drugs may result in treatment failures.

We propose impactful treatment of TB by early diagnosis target-based next generation sequencing (NGS) to cover mutations to first- and second-line drugs to determine resistance to drugs other than rifampicin (RIF) to guide appropriate personalized regimens in high risk TB groups. Bioinformatics based analysis of sequence data allows identification of resistance causing mutations.

Targeted genetic sequencing of MTB using NGS will have great value in early diagnosis of DR in individuals who are at high risk of developing resistance such as, those who have previously received TB treatment. NGS based testing will give comprehensive genotype information for new drugs including bedaquiline and delaminid. This would further guide personalized personalized treatment of drug resistant TB at the time of treatment initiation improving treatment and management outcomes.

Key words:

Tuberculosis; Drug resistance; bioinformatics; mutations

Increased risk of leukemia in patients of Xeroderma Pigmentosum is caused by mutator phenotype associated with purine mutations

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Xeroderma pigmentosum (XP) is an autosomal recessive genetic disorder caused by impaired Nucleotide Excision Repair (NER) pathway characterized by sensitivity to exposure to genotoxins causing bulky DNA adducts, such as for example UV-light. A growing body of evidence indicates that NER proteins may also participate in the removal of bulky lesions that are produced by the normal cell metabolism and endogenous mutagens. XP patients are known to harbor a 20-fold increased risk of internal solid cancers and leukemia. This may be associated with increased mutagenesis in the internal cells of the body resulting from inability of NER deficient cells to repair bulky lesions induced by endogenous mutagens. In order to study mutational processes in internal cells of the body of XPC patients we performed WGS of 6 leukemias and 2 internal solid tumors. We have detected a very high mutation rate in all tumors, which was ~50 fold higher than age and tumor type matched sporadic cancers (200'000 mutations in 8 tumors). More than 90% of mutations in all XPC tumors were corresponding to COSMIC Signature 8 and were not different between tumor types. Compatible with specific deficiency of Global Genome NER, mutation rates on the untranscribed stand were on average 2 fold increased as compared to the transcribed strand, and more than 5 fold – in highly expressed genes. Moreover, this analysis indicates that mutations occur from purines (predominantly from guanine and to a lesser extent from adenine). This study highlights the impact of familial cancer syndromes into understanding of mutational processes in somatic tissues and cancer.

ABSTRACTS OF
INVITED LECTURES IN ICAB-2019

Congenital heart defects- genetic biomarkers, technologies and challenges

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Heart development is a complex process. Heart development requires intricate cellular differentiation involving morphological and functional changes during the embryonic and postnatal periods. This process is marked by distinct changes in gene expression profiles in both cardiomyocyte and non-myocyte components that must organize into an intact structure. Studies have shown that the establishment of the heart necessitates elaborate control of gene expression patterns in a temporal and spatial manner. New sequencing technologies, combined with bioinformatics and computational tools, have allowed the scientific community to appreciate the great complexity of the cardiac transcriptome. The cardiac regulatory networks are constantly updated and enriched, and lots of biomarkers have been identified, which promoted the understanding of the complex molecular mechanisms in developmental processes and potentially allowed stratified healthcare and therapeutic intervention. NKX2.5 interacts with the T-box family of transcription factors and is involved in the activation or repression of gene expression required for the differentiation of numerous cardiac structures. Faulty regulation of transcriptional programs results in both congenital heart diseases and adult cardiac diseases that have a developmental basis.

In Pakistan, there are 4 in 1000 patients, the patient numbers are increasing and not documented appropriately. Mostly these patients belonged from rural areas, hence remain misdiagnosed and untreated, that leads to a higher mortality rate.

In this presentation, genetic biomarkers and timeline for the genetic discoveries will be discussed in global and regional context. Previously used techniques like linkage analysis and advanced techniques like GWAS will be discussed. Research conducted on congenital heart diseases from our Paediatric Genetic Research Group (PGRG) in the last decade will also be discussed.

Computer Aided Drug Designing: Past, Present and Future

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Humans have devised several methods in an attempt to fulfill this apparently beyond achievable task. From using herbs and products of various life forms to partial purification and preparation of admixtures of biologically active substances, many lives have been saved and many sacrificed during this course. This hit and try method in turn leads us to the evolution of natural product chemistry to purify and characterize compounds of therapeutic potential. For better or worse, extensive and stringent criteria are in place to verify the claims of the clinical importance of these substances. This results in the extensive delay in the availability of these compounds from laboratory to the industry and subsequently to the masses. However, akin to many other arenas of science, science of drug discovery progress as a function of tireless and close to miraculous efforts of innumerable workers. As a product of this evolution, a new offshoot emerges commonly referred to as Computer Aided Drug Designing (CADD). Cutting short, augmented with the better understanding of diseases at molecular resolution and mind boggling advancements in the computer hardware and software, CADD not only has reduced the time of identification and characterization of biological active substances to noticeable scale but significantly improved the success rate of transforming this all important molecules from the compounds of interest to active drugs. This consequently squeezes the research and development cost and time of drug development for the industry, the effect of which resultantly translated to the masses. Futuristically, linking the CADD with the advancing knowledge generated through systems biology may lead to the development of drugs that could be intrinsically resistant to the resistance developed by infectious agents and cancer tissues.

Stereoselective Modification of Dydrogesterone in Green Friendly Environment

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Microbial transformation is one of the well-known methods to obtain biologically active compounds in which microorganisms act as chemical reagents. The vast amount of work in this area has been stimulated by the medicinal importance of steroids, and the desire to develop new drugs with new or improved pharmacological properties. Various kinds of steroid modifications, such as hydroxylation, epoxidation, dehydrogenation, oxidation, reduction, hydrolysis and acetylation are now routinely performed on industrial level using a wide variety of microorganisms. Many of these reactions can not be achieved by means of conventional chemical synthesis. Through microbial reactions, many novel intermediates for the synthesis of new steroid pharmaceuticals have become available.

Dydrogesterone (**1**) is a synthetic hormone, similar to the naturally occurring sex hormone, progesterone. It is a familiar drug, used to treat premenstrual syndrome, infertility and endometriosis. Incubation of dydrogesterone (**1**) with *Gibberella fujikuroi* has afforded various metabolites using standard two-stage fermentation protocol with interesting biological activities. Structures of these metabolites were deduced through modern spectroscopic techniques.

Key words: Microbial transformation, Dydrogesterone, *Gibberella fujikuroi*

Antinematodal potential of selected medicinal plants

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Nematode infections are chronic illnesses both in human beings as well as in cattle. Nematodes infect livestock and crops, affecting food production with a resultant economic impact. Medicinal plants belong to the oldest known health care products that have been used by human all over the world in the form of folk medicines or traditional medicines or ethno medicines. The use of herbal drugs extracts and their remedies have significantly increased throughout the world and the green revolution in terms of herbal medicines has now achieved astonishing popularity against the increasing use of synthetic medicines. In present study antinematodal activity of a methanol, hexane, chloroform and water extracts of *cydonia oblonga and Juglans Regia* were evaluated. *Caenorhabditis elegans*, a free living nematode was used as in vitro model in the study. A suspension of worms was treated with the extracts. After 24 hours of incubation activity was assessed in terms of number of worms exhibiting motiliy. In case of *cydonia oblonga* highest antinematodal activity was observed in water extract of leaves which is 100% while in case of stem highest antinematodal activity was observed in hexane extract of stem which is 98%. But in case of *Juglan regia* the hexane extract of husk had highest Antinematodal activity 95% *C.elegans* were died at tested concentration. No activity was observed in methanol extract of *juglan regia* fruit against nematode.

Novel nonsense mutation in *FERMT3* causes LAD-III in a Pakistani family

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Leukocyte Adhesion Deficiency-III (LAD3) is an extremely rare primary immunodeficiency disorder, transmitted with autosomal-recessive inheritance. It is caused by genetic alteration in the *FERMT3* gene, which leads to abnormal expression of kindlin-3. This cytoplasmic protein is highly expressed in leukocytes and platelets, and acts as an important regulator of integrin activation. LAD3 has features like bleeding syndrome of Glanzmann-type and leukocyte adhesion deficiency. *FERMT3* mutation(s) have not been well characterized in Pakistani patients with LAD3. In this study, an infant and his family of Pakistani origin, presenting with clinical features of LAD, were investigated to determine the underlying genetic defect. Targeted next generation sequencing (TGS) and Sanger sequencing were performed to identify and confirm the causative mutations, respectively, and their segregation within the family. A novel, homozygous *FERMT3* nonsense mutation (c.286C>T, p.Q96*) was found in the proband, and its co-segregation with LAD3 phenotype within the family was consistent with an autosomal recessive inheritance. Both parents were carriers of the same mutation. This family was offered prenatal diagnosis during first trimester of the subsequent pregnancy; the fetus carried the variant. In conclusion, our study is the first report to identify the novel homozygous variant c.286C>T, p.Q96*in the *FERMT3* gene, which might be the causative mutation for LAD3 patients of Pakistani origin.

Keywords: Primary immunodeficiency, Leukocyte adhesion deficiency type III, Targeted next generation sequencing, *FERMT3* gene, Mutation screening.

Draft genome sequence of a novel *Bacillus* glycinifermentans strain having antifungal and antibacterial properties

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Objectives: *Bacillus* spp. have been used as biocontrol agents against soilborne pathogens because they produce secondary metabolites that exhibit a wide range of antibacterial or antifungal properties. In this study, a novel strain of *Bacillus* glycinifermentans sp. (JRCGR-1) was identified and its genome was sequenced and annotated. The genome was explored for putative genes involved in antimicrobial activity.

Methods: Whole-genome sequencing was performed on an Illumina NextSeq 500 platform. Read quality was checked by FastQC, paired-end reads were trimmed using Sickle, and de novo assembly was performed using SPAdes v.3.11.11. QUAST 5.02 was used to assess the quality of contigs and scaffolds. Finally, the assembled scaffolds were annotated by Prokka v.1.13. Genes involved in antimicrobial metabolite biosynthesis were predicted using antiSMASH. Virulence and antimicrobial resistance genes were predicted using BacWGSTdb and the Comprehensive Antibiotic Resistance Database (CARD), respectively.

Results: The genome of *B*. glycinifermentans JRCGR-1 was 4 700 692 bp in size with a G + C content of 45.52%. Final assembly of the genome resulted into 84 contigs and 83 scaffolds (>500 bp length). Overall, the genome comprises 5174 genes, 32 tRNAs, 4 rRNAs, 1 tmRNA and 92 misc_RNAs. Eleven putative gene clusters responsible for antimicrobial metabolite biosynthesis were identified, including genes for biosynthesis of non-ribosomal lipopeptides and polyketides. Virulence and antimicrobial resistance genes were also identified in the genome.

Conclusion: The presence of antimicrobial resistance genes in the genome of *B*. glycinifermentans JRCGR-1 makes it a potential biocontrol agent against soilborne pathogens.

Genome Editing Using CRISPR/Cas9: An Introduction.

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The CRISPR-Cas9 system is a powerful tool for genome editing that uses a small gRNA (20 nt long) to guide Cas9 protein to a specific sequence to splice and disable a gene. This simple RNA guided genome editing technology has become a revolutionary tool and has many innovative applications in different fields of biological sciences. Here we briefly introduce the CRISPR-Cas9 mediated genome editing method, summarize the recent advances in this tool, and discuss their implications. Targeted gene knockout using the CRISPR-Cas9 system has been established in many animal and human cell lines and also in different plant species. The targeting efficiency of Cas9 has been improved by optimizing its expression that helped reduce 'off-targeting'. We also discuss off-target effects and the constraint that the Protospacer-Adjacent Motif (PAM) puts on CRISPR-Cas9 genome engineering. To address these problems, a number of bio-informatic tools are available to help design specific gRNAs, and new Cas9 variants with high fidelity and alternative PAM specificities have also been engineered. Recently, Anzalone et al. (2019) have reported a new improved CRISPR-Cas9 system called 'prime editing' that involves a longer guide RNA called prime editing guide RNA (peg RNA) and a Cas9 fused to a specific reverse transcriptase enzyme to help reduce off-targets. Owing to these recent efforts, the CRISPR-Cas9 system is becoming a revolutionary and flexible tool for genome engineering.

Pan genomic Data files processing, extraction and handling through various tailor made python scripts.

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"Bacterial genomic analysis for multiple genomes through various web based and standalone applications investigate pan genomic structure, phylogenetic attributes, horizontal / vertical transfers and genes migration pattern etc. These tools find the virulence related genes evolution and mechanisms involved in bacterial resistance or to enable bacteria survive in the hostile environment. The challenge is to extract meaningful and required / specific data type and process huge data files generated during pan genomic analysis without hassle. Our customized python scripts extracted required information from the data files generated by Pan genomic analysis pipeline (PGAP) or reciprocal blastp and the extracted information can be used to build tables. The objective is to shape data in simpler and understandable tables and figures. The extracted data obtained through scripts converted in the tabular form from big data files for example blastp results with specific identity percent or PGAP output files for example 1.Orthologs_Cluster file and tree files. The tailor made scripts enable user to obtain targeted information and present it as Figures or Tables. The scripts are not limited to PGAP data and can be used for other genomic data type with no or little modification."

Development and commercial formulation of antagonistic yeasts for the biological control of postharvest fungal diseases of fresh fruits

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Several methods are used to control postharvest losses of fresh fruits which are mainly caused by fungal pathogens. The use of environment friendly antagonistic yeasts as bio-fungicides to control such diseases is one of the best choices. In the present study, therefore, we have isolated several fungal pathogens and environment friendly yeasts associated with the fresh fruits such as kinnow, apples, pomegranate and peach and identified them on the basis of their macroscopic and microscopic features and finally by DNA sequencing of ther PCR amplifed products of ITS regions. Antagonistic activity of several yeast cultures was tested against fungal pathogens and the yeast strains that showed significant anagonisic activity were identified as *Candida melibiosica*, *Debaryomyces hansenii*, *D. nepalensis* and *Meyerozyma caribbica*. During commercial formulation in laboratory trials for all antagonistic yeast strains *M. caribbica* produced highest biomass as compared to *C. melibiosica*, *D. hansenii* and *D. nepalensis*. Investigating eeffects of various factors such as pH, medium and agitation rates on the biomass production of *M. caribbica* showed that this strain can produce up to 18 grams per liter of yeast biomass at 15rpm agitation rate and controlled pH 5 in modified YM medium. These results are comparable with baker's yeasts (*Saccharomyces cerevisiae*) which can produce up to 20 gram per liter biomass at optimized conditions.

NMR-based Metabolomics as a tool to study biotic stress in grapevine

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It is accepted that living organisms are capable to produce compounds of complex and diverse structures and functions, known as primary and secondary metabolites. These metabolites are not only vital for the living system to perform the normal physiological functions but also crucial to deal with numerous biotic and abiotic stress factors, particularly in plants. The unbiased analysis of *all* these metabolites can provide useful information to define living systems at the level of genes, transcript, and proteins. The approach aimed towards providing a comprehensive qualitative and quantitative overview of all the metabolites present in a system at a particular time is termed as 'Metabolomics'. The data generated from this technology can not only be used for the phenotypic characterization of an organism but also provide aid to various metabolic engineering studies, to understand stress physiology, identification of novel metabolites, quality control, and prediction of pharmacological activities. With the advancement in the field of analytical chemistry, more powerful and sophisticated tools (like mass spectrometry and nuclear magnetic resonance) for such chemical analyses have been introduced. Chemical analysis techniques applied to metabolites profiling should be unbiased, rapid, and reproducible, while requiring only simple sample preparation. Many platforms are being used for the high throughput analysis of metabolites, but vary according to their sensitivity. This study will present results of an attempt to develop a method to study the metabolic response of plants under biotic stress.

Keywords: Biotic stress, Grapevine, NMR, Metabolomics, Multivariate Data Analyses

ABSTRACTS OF
SESSION LECTURES IN ICAB-2019

Association of vitamin d with type 2 diabetes mellitus in Karachi, Pakistan

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Hypovitaminosis D is a common modality in the world populations and is believed to be associated with the onset of type 2 diabetes mellitus (T2DM). In this study status of vitamin D along with other parameters in type 2 diabetic patients were assessed. Vitamin D (VD), Calcium (Ca), Phosphorous (Ph), random blood glucose (RBS) and HbA1c levels were measured in 192 diabetic subjects. Hypovitaminosis D was prevalent in diabetic patients while, Ca and Ph levels were found in normal physiological ranges. RBS and HbA1c showed negative association with VD level in vitamin D deficient and diabetic patients with good glycemic control.

Identification of Mutations in Gene *BRCA1/2* in Breast Cancer Cases from Balochistan, Pakistan

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ABSTRACT

Breast cancer is one of the leading causes of death, accounting for about 25% of all cancer cases among women worldwide. *BRCA1/2* genes are highly susceptible genes for both breast and ovarian cancer and account for 15-20% of all the hereditary breast cancer cases. The present study was aimed at identifying mutation in *BRCA1/2* in breast cancer patients in Balochistan. A total of 100 blood samples including 50 breast cancer cases and 50 normal subjects were used to determine genetic variants in *BRCA1* and *BRCA2*. DNA was extracted from the blood samples through an inorganic method. Primers were designed for all the coding exons of *BRCA1/2* genes using the Prime3 software. All the amplified products were sequenced on 3100 ABI prism DNA sequencer using the Big Dye Terminator Cycle Sequencing Kit. The results of DNA sequences were analyzed for the genetic variants by comparing the sequences of the exons with the normal sequences of *BRCA1/2* genes (ENST00000357654.7) through ENSEMBLE genome browser. Nine variants were identified in *BRCA1* and four were identified in *BRCA2.* In case of *BRCA1*, six missense substitutions (p.Asp343Tyr, p.Gly393Asp, p.Ser561Phe, p.Ser616Phe, p.Pro871Leu and p.Ser1613Gly) two frameshift (p.Ser423fs and p.Gly1770fs) and a nonsense mutation (p.Glu1250X) was identified. In case of *BRCA2* gene, all the four variants were missense substitutions (p.Glu58Lys, p.Asp99Asn, p.Asp104Asn and p.Pro999Gln). The current study revealed 13 different genetic variants including missense, nonsense and frameshift mutations in gene *BRCA1/2* in breast cancer cases from Balochistan. The data reported here will be valuable addition for the future genetic screening of the breast cancer patients of Balochistan.

Keywords: Breast cancer, Missense mutation, Nonsense mutation, Heterozygous, Gene *BRCA1/2*.

Investigation of newly bacteriologically positive Pulmonary Tuberculosis TB by using GenXpert Instrument among the District of Khairpur Mir's Sindh Pakistan

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Background: The causative agent of Tuberculosis is mycobacterium tuberculosis, and it's a contagious disease spread from person to persons. According to WHO guide line one patient is suffering from the TB, he may responsible for up to 16 other TB patients annually if he will not treated at the stage of pre-diagnosed. So it's serious threat to public health and huge challenge for us to diagnose early at the stage of presumptive in order to safe local community.

Aim: Diagnose of newly pulmonary bacterial positive patients using advance Technology GenXpert tool for the detection of *Mycobacterium* Tuberculosis.

Materials and methods: *Mycobacterium* Tuberculosis (MTB) was amplified by using sophisticated real time polymerase chain reaction GenXpert sixteen modules machine made by Cepheid *GeneXpert*® USA. Samples were also received from other facilities of district. Before this test Acid Fast Bacilli AFB test was also performed for selected samples. All TB suspected samples were collected in sterile container by following standard protocols of WHO. The size of samples were collected by following the guide line of WHO, the study was conducted from January 1st to 31 December 2018

Results Discussion: Total 1578 tests were performed on the basis of TB presumptive cases in the district TB laboratory of Khairpur Mirs, among them 262 samples were smear positive and 512 samples were smear negative, without smear samples was performed up to 804. It was found that 7 cases was justified MTB with rifampin (Rif) resistant and 218 cases was only MTB, although smear negative Rif resistant only 2 cases were investigated and 36 cases were positive with MTB positive. Regarding the cases of smear negative 9 samples were positive with Rif resistant and 132 were found as MTB. Consequently Laboratory findings demonstrates that 1.140% was MTB with Rif resistant, 24.161% was found only with MTB positive in District Khairpur including almost all the public TB Medicare facilities. **Conclusion:** its determined that cases of TB in the district are higher and its need to eliminate this contagious from the targeted areas.

Keywords: Rif, Rifampin TB, Tuberculosis, MTB, Mycobacterium Tuberculosis

Participation of seam nuclei in regulation of coherent eeg relations under conditions of experimental retinal dystrophy

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In the present work, in conditions of chronic experiments on rabbits, the effects of electrical stimulation of weld nucleus neurons on the formation of coherent (Coh) connections between EEG of the central structures of the visual analyzer in the background of experimental retinal dystrophy are considered. It is shown that in the background of experimental retinal pathology there is a significant decrease in average values of Coh coefficients between analyzed structures and their redistribution in frequency bands of EEG. The electrical stimulation of weld nucleus neurons in the background of pathology largely restores the background picture of the distribution of coherent relations. The effects of stimulation of the seam nuclei that occur during the period of subsequent stimulation, are long-lasting and have a cumulative effect.

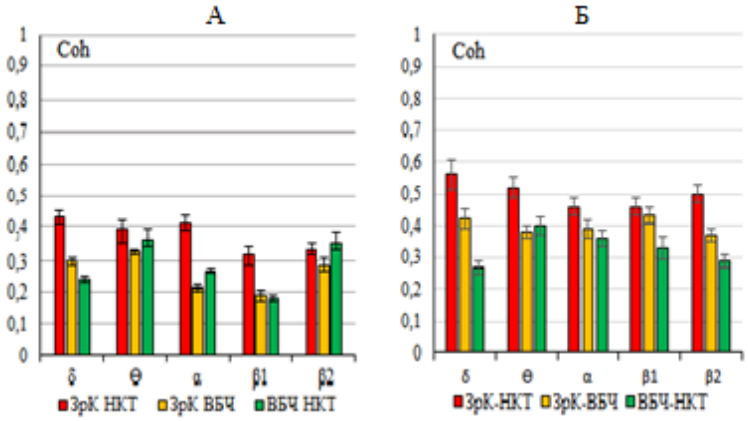

Fig. 1. Distribution of averaged Coh coefficients between visual analyzer structures. A - in the background of experimental retinal dystrophy; B - during the period of follow-up of electrical stimulation of weld nuclei.

Based on the known features of the ultrastructural organization and synoptology of weld nucleus neurons, it is concluded that the features of redistribution of coherent EEG connections in the visual system under the influence of electrical stimulation of the seam are due to the non-synaptic action of serotonin on the neurons of central structures of the visual analyzer.

Role of licorice root extracts *(Radix glycyrrhizae)* and *Danae racemosa* in protecting photosystem II under oxidative stress

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It was determined the role of extracts derived from licorice roots (*Radix glycyrrhizae*) and the relict plant *Danae racemosa* in protecting the activity of photosystem II (FS II) and the absorbance of pigments Chl a (680 nm) and Chl b (645 nm) under oxidative stress caused by toxic action of Ni^{2+} (10^{-3}M). The study was conducted on 7-day seedlings of wheat (*Triticum aestivum L.)* grown at different pH (4.5; 6,8; 9.0) medium. The toxic effects of the Ni^{2+} were shown to alter the nature of the milliseconds delayed fluorescence of chlorophyll a (msec DF Chl a). Both the donor side and the acceptor side of the transport chain electron (ETC) of PS II were inactivated.

Addition of licorice extract to the medium at pH 4.5 and 6.8 caused restoration of the fast phase activity associated with the recombination process on the donor side of the ETC PS II on the 35-40%, and at pH 9, 0- on the 25% relative to the action of the $Ni^{2+.}$ The effect of the *Danae racemosa* extract at pH 4.5 and 6.8 was observed in the recovery of 50% of the activity of the fast phase of the msec of the DF Chla, and of the slow phase of the msec of the DF Chla, responsible for the stability of electron transfer to the quinone acceptors Q_A-Q_B - 2 times more with respect to nickel ions. The effect of licorice extract was found at all pH in the recovery of activity of only the slow phase msec of DF Chla.

Analysis of absorption spectra of native forms of chlorophyll (Chl) showed that at all pH of the medium, their absorbance decreased 2 times compared to the control under Ni^{2+} action. When *Danae racemosa* extracts and licorice roots were added to the acidic medium, the absorbance of Chl b at 645 nm was restored 1.5 times and Chl a at 680 nm 3 times. The results obtained at pH 6.8 showed that the absorbency of Chl b form was most effectively restored by the action of *Danae racemosa* extract. The effect of licorice was expressed in an alkaline medium (pH 9.0) and the absorbency of Chl a and Chl b forms increased 3 times.

It is assumed that the used extracts containing polyphenol compounds can scavenge free radicals formed by the toxic action of Ni^{2+}.

Metagenomic profiling of fresh water lakes at different altitudes in Pakistan

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Freshwater lakes play a crucial role in sustainability of ecosystem in the favour of communities flourishing around it. In this study, differential metagenomic evaluation of three freshwater ecosystems in Pakistan was carried out. These included Lakes Keenjhar, Rawal and Saif-ul-Muluk situated at the altitudes 21, 527, 3224 meters respectively. Former two lakes serve as water reservoirs for three major cities of Pakistan, while the high-altitude Lake Saif-ul-Muluk is located in a pristine environment at the meeting point of Himalayan and Karakoram ranges. Next generation sequencing (NGS) of metagenomic DNA of microbial communities was carried out using surface water samples from these lakes by Illumina Hi-Seq 2500 sequencing technology. Bioinformatics analysis identified *Proteobacteria* as the most dominant phylum (58-79%) followed by *Planctomycetes* (34%), *Cyanobacteria* (12%) and *Bacteroidetes* (15%). Lake Saif-ul-Muluk contained highest abundance of bacterial genera including *Vibrio, Bordetella, Pseudomonas, Burkholderia* and *Escherichia;* while *Microcystis* were found to be abundant in Lakes Keenjhar and Rawal. Non-parametric multi-dimensional scaling (NMDS) principle component analysis on genus level revealed that these ecosystems were divergent from each other and from metagenomes of lakes in the Americas. Characterization of virulence factors and antibiotics resistant genes in metagenomic contigs were also identified. Chemical profiling of Lakes Keenjhar and Rawal showed higher concentration of phosphorus which indicated eutrophication in these lakes might be due to *Microcystis* infestation.

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Functional characterization of Nematicidal 'major royal jelly protein' containing glycoproteins from Acacia honey

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Parasitic nematodes infect more than two billion people worldwide particularly in developing countries. We have previously reported nematicidal activity of natural honey using model nematode Caenorhabditis elegans. In this study, characterization of nematicidal effects of natural honey and its glycoproteins has been carried out. Chromatographically separated honey glycoproteins showed potent anti-*C. elegans* activity (LD50=100 ng proteins/μL). Honey glycoproteins with molecular masses of □260 kD and □160 kD comprised of 'major royal jelly protein-1'-containing complexes. In these complexes, MRJP1 was present in different glycosylation forms. Quantitative PCR based gene expression assays described molecular functions of *C. elegans* affected by honey and honey glycoproteins. Expression of 14 gene transcripts associated with key cellular and molecular functions including energy metabolism, cytoskeleton, cell division, transcription and translation was analyzed. Acacia honey exerted a concentration-dependent alteration of gene transcripts involved in the citric acid cycle (mdh-1 and idhg-1) and cytoskeleton (act-1, act-2, and arp6). Likewise, MRJP1-containing glycoproteins caused downregulation of arp-6 and idhg-1; and up-regulation of act-1 and mdh-1 gene transcripts. Consistent down-regulation of isocitrate dehydrogenase encoding idhg-1 gene which is among the rate-controlling enzymes of the citric acid cycle was considered as main biochemical factor involved in the nematicidal activity of honey and MRJP-containing glycoproteins. Acacia honey suppressed the expression of gene transcripts encoding actin-2, while honey glycoproteins did not. Hence, honey partly exerted anti-*C. elegans* activity by decreasing the transcription of actin-2 gene transcripts, demonstrated by a defect in the movement and egg laying. Moreover, arp-6 gene transcripts encoding actin-related protein 6 was significantly and constantly down-regulated by honey and honey proteins.

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Probiotics protect intestinal microbial dysbiosis and subsequent aero-gastric infections of *S. aureus* and *P. aeruginosa*

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Studies on intestinal microbiome and the balance in this microbial ecosystem has been of great interest in past decade. Human digestive tract has immense assortment of microorganisms and the balance in this ecological niche is dependent on several factors including gastric acidity and motility etc. Gut microbial disturbances or dysbiosis may cause an assortment of illnesses or anomalous physiological states. Infections caused by pathogens and exposure to antibiotics can change gastro-intestinal microbial ecosystem and create the opportunity for opportunists to over grow and translocate to extra-intestinal niches. ***S. aureus*** and ***P. aeruginosa*** are well known opportunists and nosocomial pathogens. They appear to have the opportunity and the ability to promote intestinal and respiratory infections. They are largely the cause of morbidity and mortality in both hospital and community settings. These pathogens remain the important cause of pulmonary infections in case of Cystic fibrosis patients with a worldwide prevalence. Although antibiotics are still an effective means of treating bacterial infections, the alarming rise of multi drug resistant bacteria has urged to seek for the new therapeutic approaches. Thus, there is a need for the development of potent antimicrobial for the effective treatment of infections. Currently *Lactobacillus* and *Bifidobacterium* species are pulling in incredible enthusiasm as health supplements due to expanded familiarity of the beneficial roles in health and nutrition. They can possibly repress pathogen colonization and modulate host immune response. They play role in keeping the gut microbial ecosystem stable by reestablishing normal microbiota. There is evidence that maintaining balanced intestinal microbial ecosystem can antagonize the access of respiratory and enteric pathogens.

KEYWORDS
Gut microbiome; Gut-Lung axis; Microbial dysbiosis; Probiotics

Fabrication and characterization of antibacterial biodegradable polymeric nanofibers

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Electrospun nanofibrous membranes has gained great focused in medical research due to its biocompatibility and biodegradability. During present study sustainable electrospun nanofibers were fabricated via biodegradable synthetic polymer PVA (poly vinyl alcohol) loaded with antibiotic Levofloxacin. Control (PVA) and Drug loaded (PVA/LVF) nanofibers were synthesized through electrospinning and morphologically characterized through scanned electron microscopy (SEM) that showed average diameter of 146.24±44.024 nm and 184.79±41.94 nm respectively. In FTIR spectrum PVA showed characteristic peaks at 2910 cm^{-1} and 2941 cm^{-1} comes in the ranges of 2800 to 3200 cm^{-1}, conforms the presence of OH group of PVA. While pure drug (Levofloxacin) is showing small stretching peaks at 1143 cm^{-1} and 1327 cm^{-1}, which are very much close to carboxylic acid group. Linkage of quinolone group with carboxylic acid is causing a possible variance of peaks from pure carboxylic ranges in blended sample as the peaks were shifted from 1143 cm^{-1} to 1141 cm^{-1} and 1327 cm^{-1} to 1328 cm^{-1}. Further antibacterial susceptibility was checked against Escherichia Coli (E.coli) for different times of incubation and maximum zone of inhibition was observed after 72 hours that is 29±0.25 mm as compare to 24 h and 48 h. Drug release profile was measured by spectrophotometric method up to 72 hours in PBS solution. Initially nano fibers showed burst release of drug up to 24 hour, whereas later on sustain release behavior was observed up to 72 h. Above results suggested that drug loaded nanofibers membranes could be used in synthesis of sutures, wound patches bandages and other biomedical applications.

Keywords: Electrospinning, PVA, Levofloxacin, Bacterial susceptibility, Sustained drug release.

Strategic Classifications and Valuable Products Preparation from Leather Solid Wastes: A Step toward Sustainability of Leather Sector

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Abstract:

The conversion of one metric ton of salted hides/skins into leather produces approximately 300kg of final finished leather along with 250Kg of chromium containing solid wastes, 350Kg of chromium free waste and about 100 Kg goes in waste water. In detail, these solid wastes generated from tanneries include hairs (keratin) wastes, skin trimmings, fleshings, buffing dust and chrome shavings. In most of the developing countries, these wastes are thrown out in open places, damped in sites or incinerated. Such type of waste management may cause bad smell, increase carbon level in air and risk of inclusion of toxic chemicals in food chain and potable water. Being a protein rich material these wastes may be used for the preparation of very valuable products. Although many efforts have been done to convert these materials into various products but most of them are costly, random, incomplete and non eco-friendly. Therefore we emphasized on precise classification and proper strategies to convert leather solid wastes into valuable products. In our research chrome containing wastes have been recycled in leather processing after converting them into appropriate products. Safe and non-risky waste has been converted into products of food chain. Chrome free but risky wastes have been utilized for energy generation. In this way economical, eco-friendly, proper valuable products from solid wastes have been obtained to ensure the sustainability of leather sector.

Metagenomic Analysis of Drinking Water Samples Collected from Treatment Plants of Hyderabad City and Mehran University Employees Cooperative Housing Society

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The quality assessment of water, supplied to the end user, is an essential part to assess the physical, chemical and biological status of water, which impacts on human health. For the quality assessment of drinking water treatment plants and distribution systems of Hyderabad city and Mehran University of Engineering and Technology, Jamshoro, Pakistan, 13 surface drinking water samples were collected from three treatment plants, two of Hyderabad city, including WASA treatment plant and its distribution system (n=5), Hala Nakka treatment plant and its distribution system (n=6) and Mehran University Employees Cooperative Housing Society (MUECHS) treatment plant and its distribution system (n=2). Physicochemical parameters of all drinking water samples were in the range compared to EPA and WHO guidelines, except in L-12 sample. Notably, no free-chlorine was detected in all samples. In metagenomics analysis, targeting V3-V4 hyper-variable region of 16S rRNA gene, by using QIIME2 showed high bacterial prevalence in all samples. On average, 348 OTUs were observed per sample. Among all samples, treated water sample from the Hala Nakka Treatment Plant (HNTR) was most diverse sample in bacterial composition (Shannon 7.51 and Simpsons reciprocal indices 0.98). Overall, Proteobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia, and Actinobacteria were the five most abundant phyla (relative abundances of 43.6, 37.9, 8.5, 2.5, and 2.4 percent, respectively). Notably, Cyanobacteria are well known toxin producers which effect the human, and animal health. At genus level, *Flavobacterium* (4.86%) and *Aquirestis* (3.77%) were the most abundant genera. Functional predictions, based on 16S rRNA gene by PICRUSt, predicted 6909 KEGG orthologies, relating to 245 KEGG pathways. Among the predicted pathways of KEGG orthologies, pathways to human infections were also found. In conclusion, this study gave a deep insight of bacterial contamination in drinking water samples of Hyderabad City and MUECHS treatment plants and water quality status in Hyderabad and Mehran University of Engineering and Technology.

Whole genome sequencing and assembly of Pakistani cattle breeds (*Bos-indicus*) to provide baseline big data for indicine genomic selection and dairy traits enhancement.

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The primary goal of cattle genomics is the identification of genome-wide polymorphism associated with economically important traits. The bovine genome sequencing project was completed in 2009. Since then, using massively parallel sequencing technologies, a large number of *Bos taurus* cattle breeds have been resequenced and scanned for genome-wide polymorphisms. As a result, a substantial number of single nucleotide polymorphisms (SNPs) have been discovered across European *Bos taurus* genomes, whereas extremely less number of SNPs are cataloged for *Bos indicus* breeds. In this study, we performed whole-genome resequencing, reference-based mapping, functional annotation and gene enrichment analysis of 20 sires representing eleven important *Bos indicus* (indicine) breeds of Pakistan. The breeds sequenced here include: Sahiwal and Red Sindhi (tropically adapted milking breeds), Bhagnari, Dajal, Dhanni and Lohani (major draught breeds); Achai and Gabrali (dairy and light drought purposes); Tharparkar and Cholistani (tropically adapted dual-purpose breeds). A total of 17.4 billion QC passed reads produced using BGISEQ-500 next generation sequencing platform to generate 9 to 27-fold genome coverage (average ~16×) for each of the 20 sequenced sires. A total of 67,303,469 SNPs were identified, of which 3,850,365 were found novel and 1,083,842 insertions-deletions (InDels) were detected across the whole resequenced genomes (491,247 novel). Comparative analysis using coding region SNPs revealed a close relationship between the best milking indicine breeds; Red Sindhi and Sahiwal. On the other hand, Bhagnari and Tharparkar being popular for their adaptation to dry and extremely hot climates found to share most SNPs. Functional annotation identified a total of 3,194 high-impact (disruptive) SNPs and 745 disruptive InDels (in 275 genes) that may possibly affect economically important dairy and beef traits. Functional enrichment analysis was performed and revealed that high or moderate impact variants in wingless-related integration site (Wnt) and vascular smooth muscle contraction (VSMC) signaling pathways were significantly overrepresented in tropically adapted heat tolerant Pakistani-indicine breeds. On the other hand vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF-1) signaling pathways were found over-represented in highland adapted Pakistani-indicine breeds. Similarly, the ECM-receptor interaction and Jak-STAT signaling pathway were significantly enriched in dairy and beef purpose Pakistani-indicine cattle breeds. The Toll-like receptor signaling pathway was significantly enriched in most of the Pakistani-indicine cattle. Therefore, this study provides baseline data for further research to investigate the molecular mechanisms of major traits and to develop potential genomic markers associated with economically important breeding traits, particularly in indicine cattle.

Vector Borne Dengue Disease using R/S Oncological Treatment

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Vector Borne dengue fever is recorded as an urban seasonal human disease as it spreads easily to urban morphological contexts, because of the uneven increase of urban population and infectious diseases as a result of climate change. Dengue epidemic cases related to climatic factors are helpful to monitor and prevent the transmission of dengue fever. We carry out the epidemiological study to investigate the dengue fever development and prediction in the Karachi city. This study described that the oncological treatment by statistical (R/S) analysis of the dengue epidemics from January 2001-December 2018, based on the urban sustainability development, and climatic parameters including temperature and ENSO respectively. Climate parameters are shown that the R/S value revealed a persistency($1<D<1.5$), R/S analysis also confirmed the anti-persistent($1.5<D<2$)of dengue for months of September to November and these months has normality ADT ($A=2.156$ to 2.365) itemized the robust indication of the complexity of data.

Keywords: Dengue Epidemic, Urban human arboviral disease, Urban Morphology, Oncological treatment.

Sustainable Growth in Production of Nutritious Foods Following the Persistent Footprints of Malnutrition in Cereal-Based Subsistence Dietary Foods in Pakistan

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Maximizing agricultural production for assuring food and nutritional requirements of rapidly growing human populations is a major sustainability challenge of this 21st century. The United Nation's Sustainable Development Goals (SDGs) call for sufficient quantity of food and nutritious also to feed 9.7 billion people by 2050. Furthermore the increase in food production is to be raised by 70 % over the current level of productivity under the climate change barbarism. In this context, food production, supply and processing of food products makes agriculture and intuitively 'straight forward' Sector in the realm of food and nutrition security. In Pakistan, it is matter of great concern that 100 million people are suffering from acute micronutrients malnutrition, despite consumption of 126 kg wheat flour/capita/annum. The reason being the cereal including wheat foods are poor in dietary nutrients and people have a little excess to fruits, vegetables and meet products, to meet their body needs. Among South-Asian countries, more than 100 million inhabitants of Pakistan are subsisting on cereal-base diet, which are poor in essential dietary nutrients. The obnoxious effects of malnutrition take a heavy toll on health of infants, children and women of reproductive age, particularly in the form of high morbidity and mortality rates. Resultantly, about 44% children under 5 years are stunted, 32% underweight and 15% wasted, while, 54%, 40% and 39% are at risk of deficiency of vitamins (A&D) and zinc nutrient, respectively. It is astonishing to note that even 97% of children are suffering from micronutrients in the big cities. It is pertinent to mention that child is ought to be properly nourished for first 1000 day of one's life and beyond this age, the irreversible loss cannot be rejuvenated at any cost. Furthermore, the footprints of malnutrition also tolls to US$ 7.6 billion annually (equals to 3% of GDP), including US$ 2.1 billion loss in workforce, US$ 1.0 billion due to anemia and diarrheal disease, US$ 2.24 billion from zinc deficiency syndrome alone, and US$ 2.1 billion due to iron deficiency. Moreover, today's crop commodities also possess on an average, half of the contents of macro-and micronutrients such as iron, zinc, copper, manganese and selenium compared to those farm produce during 1940s. In the wake of climate change, the prevailing situation will be worsened to greater proportion over a longer period. In the contemporary world of human nutrition, there is growing body of evidence that deficiency of dietary nutrients could be achieved through agriculture-nutrition nexus, i.e., genetic manipulation on the analogous to Green Revolution. The biofortified high zinc wheat variety "Zincol-2016" has been released for general cultivation. It contains more than 37 microgram zinc per gram (+12 over baseline, in conventional varieties). This variety is highly competitive and high yielding, resistant to diseases including stem rust Ug99. This variety can be cultivated over years without occurrence of erosion in the genetic makeup as well as reduction in the nutrient contents in the grains. The consumption of biofortified wheat provides sufficient quantities of dietary nutrients viz., zinc, iron, calcium, phosphorus and potassium to suffice human requirement.

Keywords: Food security, Nutrition security, Biofortified zinc wheat, Mitigating malnutrition, Human health

Preventive Role of Gabapentin in Transmission of Inflammatory Nociception in Acute and Sub-chronic Inflammatory Models of Rat

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Nociception can trigger an increase in the expression of different genes and their product that can mediate or modulate nociception related changes in the brain. There are a number of experimental models of inflammatory pain provide evidence that nociception results in increased expression of early immediate genes, c-fos that are associated with a hyper neuronal activity. It has also been proven that ɣ-aminobutyric acid (GABA), an amino acid neurotransmitter, can involve in inhibitory control of nociception and mediate sensory inputs at the spinal cord level. GABA itself influences the expression of certain genes including c-fos in different disease conditions such as seizures. The present study was designed to investigate the effect of Gabapentin, an analog of GABA, on the expression of c-fos in carrageenan induced-acute inflammation. Our results have been shown that in the animal receiving the only carrageenan, there was a marked increase in the expression of the c-fos gene in different brain areas with different intensity. Results show that Gabapentin treatment has a potency to inhibit the expression of the c-fos gene, which the hallmark of neuronal hyperactivity during inflammatory pain. Gabapentin also prevented the development of other pain-related behaviors, such as paw withdrawal latency responses; we observed that pain scores were not statistically different from baseline. This study suggests that modulating the neurotransmission of nociception can suppress effectively the level of nociception associated with acute inflammation with reducing side effect associated with the extended use of conventional anti-inflammatory drugs.

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Structural investigation of human dectin-1 receptor; A novel gateway in drug discovery

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Stimulation of C-type lectin domain of human dectin-1 receptor by fungal β-glucans causes conformational changes in its cytoplasmic domain which initiates various cellular responses mediated by downstream signaling components. We aimed to build the three-dimensional structures of the cytoplasmic domain as well as C-type lectin domain of humanDectin-1along with their potential ligands through homology modeling. The overall three-dimensional fold of cytoplasmic domain was found to consist of mixed β-sheet whereas,in case of C-type lectin domain antiparallel β-sheets flanked byα-helices were observed. Protein-protein docking strategy was utilized to monitor key interactions between cytoplasmic domain of dectin-1 receptor and PKCδ, as a prime regulator of Dectin-1 signaling. The interface was observed to have both hydrophilic and hydrophobic amino acid residues maintaining crucial contacts between the two proteins. The given three dimensional structural information can be implicated in structure-based drug designing to discover potential immunomodulators that can interfere with the immune responses and phagocytosis during inflammatory and infectious conditions.

Targeting membrane associated proteins in understanding molecular mechanisms and treatment of Alzheimer's Disease.

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Alzheimer's Disease (AD) is a major health concern in modern society. Currently 24 million people across the globe are struggling against the symptoms of AD and this number is expected to double every twenty years. Various molecular mechanisms and interacting networks are altered in AD due to aberrant expression of proteins. Membrane proteins that constitute 30% of total cellular proteins are the major drug targets of therapeutic approaches used in AD.

The present study aims to identify the role of membrane associated proteins, their interactions and perturbations pertaining to AD and highlight their role as a drug target in the treatment of disease.

Comparative brain membrane proteomics of healthy control and AD subjects was performed to gain insight into the molecular mechanisms and interacting pathways. Two-Dimensional Blue Native PAGE (2D-BN-PAGE) followed by Nano LC-MS/MS analysis was performed of proteins obtained from the brain tissue of healthy control and AD subjects followed by interaction analysis by STRING databases and Ingenuity Pathway Analysis (IPA). Multiple membrane proteins chiefly FRIH, SLC4A4, ATP6V0A1SLC1A2, MGST1, ACTN1, HSP90AA1, GAPDH, PLP1, STXBP1 and HS90B were found to be differentially expressed in AD brain with altered interacting networks and molecular mechanisms suggesting their contribution in the development of AD.

Further understanding of membrane proteins combined with molecular docking studies could help to unravel the role of proteins associated with membrane as a drug target and in developing new therapeutic opportunities to prevent and retard the progression of AD

Key words:

Alzheimer's Disease, membrane proteins, Blue Native PAGE, protein networks

Study of BCR-ABL gene and cytogenetic analysis of chronic myeloid Leukemia patients

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Chronic myelocytic leukemia is a disease of the hematopoietic stem cells. CML is a type of cancer produced by the balanced translocations amongst the long arms of chromosomes 9 and 22, which are called as the Philadelphia chromosome. The study was performed on 131 CML patients. CBC of 131 patients were done, Karyotyping were conducted on 76 CML patients for the additional chromosome abnormalities and Fish (38 /131) patients. 22 samples were for resistance against Imatinib and shifted to 2nd generation Nilotinib within 01 year. Among 131 patients were enrolled, in 72 (54.96 %) were male and 59(45.03%) were female. Cytogenetic analysis results show that 53(69.53%) patients were with Ph positive. At diagnosis 61(92.42%) patients were in chronic phase, 04(6.06) were in accelerated phase and only 01(1.51) were in blast crisis. Thirty-three showed increases level of BCR-ABL fusion cells while 05 showed 0% BCR-ABL negative cells**.** 06 CML patients who shifted to from Imatinib to Nilotinib.

Prediction of Stable Tautomers through Acid Dissociation Constant: Theory vs. Experiment

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The successful prediction of tautomeric state in different conditions requires detailed knowledge about the mechanism of proton transfer as well as information about the action of the stimuli. Compounds exhibiting proton transfer are used as high energy radiation detectors, fluorescent probes, polymer protectors etc. Hydrogen bonding and proton transfer reactions are two major mechanisms in biological signal processing. The aim of the present study is to understand and exploit pK_a values of tautomeric molecules like hydroxy Schiff bases and azo compounds (Figure 1) for the determination of correct position of H-atom and tautomeric structure in several different conditions. Ortho hydroxy Schiff bases have numerous effects in biological systems besides their well known participation in deamination reactions. The tautomerism of these molecules could be a key element but it is less obvious at which form these molecules are active.

This work is based on molecular modelling study, mainly by *ab initio and* density functional theory (quantum chemistry) on the acid-base properties of potential dyes like Arylazonaphthols and Aryliminenaphthols. Main emphasis will be given on the influence of external factors like solvent, substituents and pH. The influence of solvents as prototypes of apolar, polar aprotic and protic solvents will be treated by bulk solvation models. The substituents will be considered as electron donating and withdrawing groups at different position of the phenyl ring. The pK_a values of those Arylazonaphthols and Aryliminenaphthols will be computed which are already determined experimentally. The confirmation of experimental pK_a values with the computed pK_a values will help in assigning the correct position of H-atom as well as in predicting the stable tautomer.

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Effect of different cultivation conditions on callusogenesis and regeneration potential in the culture of mature barley embryos

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The idea of creating ideal models of agricultural plants adapted to certain agricultural climatic conditions has become quite widespread in recent years. In vitro technology allows to create specific models of artificial climate, which creates the conditions for breeding process acceleration. In addition, creating specific models of cultivation, plant studies at the tissue and cellular levels can be conducted in in vitro conditions. With the aim of studying the response of genotypes to various cultivation conditions and the dependence of these reactions on the genotypic characteristics of the plant mature embryos of 5 promising varieties of Azerbaijan breeding differing by source of origin, the number of rows in the ear, resistance to environmental factors and diseases were introduced in the culture: zoned variety Nakhchivandani, obtained in 1939 from local varieties of multi-row barley and 4 varieties that bred from various nurseries introduced from the International Center for Agricultural Research in the Dry Areas (ICARDA) as a result of years long selection work. They are: drought-and frost-resistant variety Gudratli-48, obtained from international winter barley hybrids nursery (IB-CB-WT); drought tolerant variety Jalilabad-19, obtained from the form of English double-row barley K-26788; Baharli variety, obtained from the variety sample of multi-row barley Rihani-03, capable to avoid spring and summer drought due to early maturity; drought and frost resistant winter barley variety Dayanatli obtained from the international observation nursery (IBON-VVT). Mature embryos isolated from the grains of these varieties were planted in 2 variants of Gamborg culture (B_5) : 1) B_5 + 7 mq/l 2,4-D + 2mq/l KIN: 2) B_5 + 10 mq/l 2,4-D+ 5mq/l KIN. 3 cultivation options were applied: 1) darkness + 26 ° C (control); 2) low lighting (3 thousand lux) + 26° C; 3) lighting (16 thousand lux) + high temperature (34 ± 2° C). Samples of the 2nd and 3rd options were transferred to ambient light conditions after 2 weeks of cultivation. The reaction of varieties to cultivation conditions and statistical processing of the results let conclude that all varieties belonged to different auxin type. In addition, it can be stated that, unlike the callusogenesis process, cultivation conditions had no significant effect on the realization of the regenerative potential of varieties.

Disorder of thyroid functional activity in patients with hepatitis C

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At present, viral hepatitis C (HCV) poses a serious problem for the health care of many countries of the world, including Azerbaijan. One of the little studied problems is the effect of viral hepatitis C on the endocrine system, in particular on the functional state of the thyroid gland. The liver plays an important role in the processes of metabolism, transport, storage and excretion of thyroid hormones. Thyroid hormones are known to regulate protein-synthetic liver function, while transport proteins of thyroid hormones, in particular thyroxin -binding globulin, thyroxin-binding prealbumin, albumin are synthesized in the liver. In connection with the disorder of viral hepatitis C protein-synthetic liver function, the transport and synthesis of thyroid hormones may suffer. The aim of the study is to study the functional condition of the thyroid gland in patients with viral hepatitis C.

For this purpose by using enzyme immunoassay, levels of tiroidnykh of hormones (a free form of a triyodtironin - TT4) and tiroidstimiuliruyushchy hormone (TSH) was surveyed in blood of 40 patients with hepatitis C. The control group was made by 25 almost healthy people who are not infected with hepatitis C. As a result of a research reduction of maintenance of TT3 and TT4 by 14.4% was observed (1.07±0.06 ng/ml; Control - 1, 25 ± 0.10 ng/ml) and 23.8% (4, 81 ± 0.28 ug/dl; Control - 6, 31 ± 0.40 μg/dl; $P < 0.01$) is reduced accordingly compared to the control. TSH concentration per 54.8% (3, 73 ± 0.27 mL U/L; Control - 2, 41 ± 0.34 ml U/l; $P < 0.01$) is increased with respect to control. It has been revealed that in HCG patients the disorder of the structural-functional state of the liver in hepatitis C leads to a decrease in the content of peripheral thyroid hormones. Increase of TSH, has compensatory-adaptation character in liver parages with hepatitis C.

ANTIOXIDANT DEFENCE SYSTEMS OF MESOPHYLL AND BUNDLE SHEATH CELLS UNDER SALT STRESS

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Salinity is one of the most important abiotic stresses limiting growth and productivity of crops. Most commonly, the stress is caused by high Na^- and Cl^- concentrations in the soil, which lower photosynthetic efficiency and may cause oxidative stress that involves an accumulation of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). In plant cells, chloroplast is a potential source of ROS production and the effect of salinity on important metabolic processes in this organelle has been well documented. Salinity-induced structural changes in chloroplasts of C_3 plants have also been reported. However, little is known about the salinity effect on the structure and biochemistry of chloroplasts in C_4 plant species.

Plants have developed an antioxidant defense system to counteract stress-induced oxidative stress. It is now believed that salt tolerance in most crop plants is associated with a more efficient antioxidant system. The antioxidative system includes both enzymatic and non-enzymatic systems. The enzymatic antioxidative system includes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), etc. The function of this antioxidant system is to scavenge the injurious radicals produced during oxidative stress and thus help the plants to survive under such conditions. A characteristic feature of C_4 plants is the differentiation of the photosynthetic leaf antioxidant defense system tissue into two distinct cell types, mesophyll and bundle sheat cells. In this study, mesophyll and bundle sheat thylakiods of maize (*Zea mays* L.) chloroplasts have been analyzed. Maize seeds germinated in the hydroponic growing medium – Knop's solution were used as the research object. Seeds first were sterilized for 15 min in 2.5% $KMnO_4$ solution, then, transferred to the filter papers and grown at 25^0C for 12 days with a photoperiod of 14 hours. The salt treatment started when the second leaf blades of the plants were fully developed by supplying 50 ml of 0 (control), 50 mM, 100 mM and 200 mM NaCl solutions every day. After the salt treatment for 5 day, the plants were analyzed. In maize, the structure of the bundle sheath cell (BSC) chloroplasts is less sensitive to salt stress than mesophyll cell (MC) chloroplasts. To elucidate the difference in sensitivity to salinity, antioxidant capacities were studied in both chloroplasts. In isolated chloroplasts, the activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were increased by salinity. Although the enhancement of SOD activity was similar in both chloroplasts, the increase in APX activity was more pronounced in BSC chloroplasts than in MC chloroplasts. Glutathione reductase (GR) activity did not detected in BSC chloroplasts, while it increased in MC chloroplasts under salt stress. Although ascorbate content increased by salinity only in BSC chloroplasts, glutathione content increased significantly in both chloroplasts, and was higher in MC chloroplasts than in BSC chloroplasts.

Biochemical and histological changes in the liver during the toxic effects of lead nitrate on white rats in the hyperthyroid model

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İntroduction

As the environmental impact of anthropogenic factors increases, pollution levels increase. Heavy metal,especially lead are among the chemicals that pollute the environment. Not only workers in the manufacturing sector? But also large numbers of people are often in contact with lead used in many industries and welfare often come to contact. This increases the risk of intoxication.

Material and method

The investigation was conducted on 18 white mice at the Scientific Research Center of Azerbaijan Medical University. The experimental animals were divided into 3 groups: In the first group 6 white mice (infected animals) were kept in *vivo* without any impact. In second group experimental hyperthyroidism model using L-thyroxine was established in 6 head rats. In the third group 3 – white experimental mice were given 2 ml 0,5 % Pb $(NO_3)_2$ solution as *per os* daily for one week following the establishment of an experimental hyperthyroid model. After this animals were decapitated and blood samples were fixed in 10 % formalin for biochemical analysis and internal organs for microscopic studies.

Results and discussions

The results of biochemical analyzes showed that the presence of major liver pathology markers in the blood of control animals was close to normal (ALT – 100, 2 + 2,3 U/l; AST – 152,2 +3,6 U/l; QF -801,5 + 18,1 U/l; MDA – 2,4 + 0,9 mkmol/l; catalaza – 10,7 + 1,6 mkat/l) these rations have changed significantly in the blood of rats in which the hyperthyroid model was created (ALT – 123, 4 + 2,8 U/l; AST –195, 2+ 3,6 U/l; QF – 900, 4+ 19, 2 U/l; MDA – 5,7±0,9 U/l; catalaza – 10, 0+ 0, 1mkat/l). In the blood of animals exposed to $Pb(NO_3)_2$ toxicity within a week after hyperthyroidism these figures were quite different (ALT- 180, 7 +2,2 U/ l; AST – 217,2 + 4,1 U/l; QF- 956,6 + 17,8 U/l; MDA – 6,8+ 1,1 mkmol/l; catalaza – 9,7 mkat/l).

Microscopic analyzes revealed a number of changes in the liver parenchyma during hyperthyroidism, including membrane damage of some parts of the hepatocytes (especially around the central vein), ureakening of the border beetween the fibers, hyperhydration and cell swelling. These pathologial changes are further exacerbated in the liver cells of animals that were intoxicated with the solution 0,5 % $Pb(NO_3)_2$ within a week after the hyperthyroid model. In the studied microscopic preparations, the number of membrane-damaged hepatocytes increased, they became swollen, fat droplets of different sizes were formed near the central vein and the number of secondary hepatocytes decreased.

Results

Based on the scientific research it is concluded that the toxic effects of lead ions are more pronounced in animals with hyperthyroid models than in intact animals. In this case? The metabolism in the liver is impaired, oxidative stress, cell membrane damage and increased permeability, hyperhydration, protein and fat dystrophy, strengthening of antioxidant defense system and etc.is being observed.

Unique but Diverse Mutational Profile of BRCA1 and TP53 in Acute Myeloid Leukemia Patients from Pakistan

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Leukemia is a malignant disorder in which mutated myoblasts and lymphoblasts proliferate abnormally and get accumulated in blood, marrow and lymph nodes. Annually 13.7 new cases are reported per 100,000 in men and woman. BRCA1 and TP53 both are the tumor suppressor genes. The proteins encoded by both genes play key role in many essential biological processes like DNA repair process, cell cycle regulation and homologous recombination. We screened acute myeloid leukemia patients from Southern, Punjab Pakistan to investigate the nature and impact of BRCA1 and TP53 mutations existing in their genomes. Different regions of both genes were amplified using DNA extracted from blood of leukemia patients as template. The PCR amplification was followed by DNA sequence analysis and bioinformatics analysis to determine the functional impact of the detected mutations on structure and function of encoded proteins. Results revealed variety (deletion, insertion, substitution and frame shift mutations) of novel mutations in BRCA1 and TP53. Various frame shift mutations generated premature termination codon and led to the synthesis of non-functional TP53 protein. Similarly many of the observed mutations disrupted the C terminal domain of BRCA1 protein making it unable to bind with DNA and regulate expression of target genes. This study suggests the role of BRCA1 and TP53 mutations in pathogenesis of leukemia and thus demands extensive exploration in future.

Keywords: BRCA1, TP53, Leukemia, Mutations

ABSTRACTS OF
YOUNG INVESTIGATOR'S PRESENTATIONS
IN ICAB-2019

Antiurolithic potential of dietary polyphenol caffeic acid in a rat model.

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Urolithiasis is the most prevalent urological disorder in the developing world. Stone formation is a multifactorial disease which is affected by a number of factors including age, sex, race, environment, diet, genetics and various biochemical factors. Kidney stones are of different chemical composition among which calcium oxalate are the most common and complex type. To date, all the available modalities for the kidney stone treatment are either expensive or less effective and are also found to be associated with certain side effects. Current literature highlights the need of a standardized herbal drug for the effective treatment of kidney stones which would be safe and economical. Various pharmacological activities of the dietary polyphenol, caffeic acid have been scientifically proved. In this regard, the current study was conducted to explore the antiurolithic activity of polyphenol caffeic acid in a rat model. Kidney stones were developed in experimental rats by the administration of ethylene glycol and rats were grouped as normal control, pathological control, standard drug control, preventive and curative treatment groups. Normal control rats received drinking water for 8 weeks along with the regular diet. Pathological, standard drug, preventive, and curative groups received 0.75% ethylene glycol in drinking water for the development of calcium oxalate stones, along with the regular diet. Standard drug group was provided Urocit-K by gavage from day 1, while preventive and curative groups received caffeic acid (20 and 40 mg/kg) by gavage from day 1 and day 14, respectively. At the end, 24 hours urine analysis was performed and rats were dissected to remove kidneys. Histopathology of kidney tissues was performed to observe effects on renal histology and crystal deposition. Real-time PCR was performed to analyze the renal expression of kidney stone related modulatory genes, i.e., osteopontin, Tamm-Horsfall, prothrombin fragment 1, and bikunin genes. The results of the biochemical analysis revealed significantly positive effects by the preventive and curative treatments with caffeic acid. Histopathological examination revealed remarkable reduction of calcium oxalate deposits with in the kidneys of the treatment group in comparison to the pathological control group. Expression studies indicated that the treatment of rats with caffeic acid resulted in downregulation of the osteopontin gene, while upregulation of the bikunin, Tamm-Horsfall and prothrombin fragment 1 genes. In brief, the results of this study propose that caffeic acid can be further explored for the prevention and treatment of calcium oxalate kidney stones.

Reference: Fauzia Yasir, Atia-tul-Wahab and M. Iqbal Choudhary. *Protective effect of dietary polyphenol caffeic acid on ethylene glycol-induced kidney stones in rats*. Urolithiasis. Apr; 46(2), 157-166, 2018.

Rapid molecular diagnostics of human pathogenic *Candida* species using specific Real time PCR based amplification of phospholipase B gene

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Invasive infections by *Candida* species are the most common cause of mortality in patients undergoing invasive procedures and immunocompromised patients. Therefore species specific primers were designed for the rapid identification and diagnosis of most common *Candida* species including C. *albicans, C. parapsilopsis, C tropicalis* and *C glabrata* from clinical samples. Rapid and species-specific real time polymerase chain reaction (Real time PCR) based molecular method was developed for the identification for said four species and developed method targeted the phospholipase B gene. In previous work we determined sequences of this gene. The results showed that the gene provides a target that could be used for the rapid and accurate identification of human pathogenic *Candida* species from the clinical samples.

Advances in *In-silico* based Predictive *In-Vivo* Profiling of Novel Potent *β-Glucuronidase* Inhibitors

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ABSTRACT

Background: Intestinal *β-glucuronidase* enzyme has significant importance in colorectal carcinogenesis. Specific inhibition of the enzyme helps to prevent immune reactivation of the glucuronide-carcinogens thus, protecting the intestine from ROS (Reactive Oxidative Species) mediated carcinogenesis. **Objectives**: Advancements in the *In-silico* based techniques is providing broad range of studies to facilitate the drug designing and development process speedily through SwissADME and BOIELD-Eggtools. **Methods**: In our designed case of study we used SwissADME and BOIELD-Egg predictive computational tools to estimate the physicochemical, human pharmacokinetics, *drug-likeness*, medicinal chemistry properties and membrane permeability characteristics of our recently *In-vitro* evaluated novel *β-Glucuronidas*e inhibitors. **Results**: Out of the eleven screened potent inhibitors, compound (**8**) exhibited the excellent bioavailability radar against the six molecular descriptors, good (ADME) absorption, distribution, metabolism and excretion properties along with P-glycoprotein, CYP450 isozymes and membranes permeability profile, on the basis of these factual observations, It can be predicted that compound (**8**) can achieve *in-vivo* experimental clearance efficiently. Therefore in future it can be a drug in market, to treat the various disorders associated with the over expression of *β-Glucuronidase*, enzyme including various types of cancers, particularly hormone-dependent cancers such as (breast, prostate, and colon cancer), while other compounds (1-7, & 9-11), have also shown good predictive pharmacokinetics, medicinal chemistry, BBB and HIA membranes permeability profiles with the requirement of slight lead optimization to obtain the improved results.

Conclusion: In the consequence *in-silico* based studies are considered to provide robustness towards a rational drug design and development approach hence to avoid the possibility of failures of drug candidates in the later stages of drug development phases. The results of this study effectively reveal the significant attributes of potent *β-Glucuronidase* inhibitors, for further experimental evaluation

Expression of htert gene and mutation analysis of its promoter in head and neck cancer in pakistan

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Head and Neck Squamous Cell Carcinomas (HNSCC) are estimated sixth most prevalent cancers in the world. Smoking, alcohol, tobacco, viral infection, and radiation exposure are the factors to cause the development of HNSCC. HNSCC accounts more in males than females. Reactivation and higher level of telomerase are the significant molecular event in HNSCC and the major cause is mutations in the promoter region of Human Telomerase Reverse Transcriptase (*hTERT)* gene. Pakistan is grouped among high risk countries for HNSCC due to late diagnosis and lack of optimum treatment.

A total of 101 samples of HNSCC undergoing biopsies were investigated to identify *hTERT* gene expression and its promoter mutations. DNA was extracted followed by PCR and nucleotide sequencing for mutation screening in the promoter region of *hTERT by* using Bio-Edit and MEGA 7.0 software and RNA was extracted followed by RT-PCR to measure the *hTERT* mRNA expression.

hTERT promoter mutations were identified in 9.9% (n=10) cases of HNSCC. These mutations showed 0-9 fold *hTERT* gene expression in the subsites of oral squamous cell carcinoma. Mutation analysis showed 5 substitutions of C>T in which 2 SNPs were on position g.4935 and 3 SNPs were on position g.5076, 2 SNPs of g 5057C>G, 1 SNP of g.4948C>A, 1 SNP of g.5026A>T and 1 SNP of g.5081G>A. Tumor development was found to be more in males than females.

TERT gene expression and its promoter mutations may represent an important tumorigenic mechanism in head and neck cancer that's need to be studied more. Selected community-based screening in high risk population will improve survival and also contribute to a better quality of life.

Keywords: Head and neck cancer, telomerase, telomerase reverse transcriptase, gene expression, mutation

Methyltrioctylammonium chloride mediated removal of lignin from sugarcane bagasse for thermostable cellulase production

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The present study was aimed to evaluate the Methyltrioctylammonium Chloride (IL) and Sodium hydroxide effect on sugarcane bagasse (SB) structure and its subsequent utilization to produce cellulase from a thermophilic bacterium *Bacillus aestuarii* UE25. The strain was isolated from a crocodile pond of Manghopir, Karachi. Ten different factors affecting IL pretreatment of SB and cellulase production by UE25 were evaluated by Plackett-Burman design and three significant factors were optimized by employing Box Behnken design. Under optimum conditions, the strain produced 118.4 IU mL−1 of EG and 75.01 IU mL−1 of BGL that corroborated well with the predicted values by the model. Scanning electron microscopy, gravimetric analysis, Fourier transform infrared spectroscopy and NMR of SB revealed removal of lignin, decrease in cellulose content and structural changes in the SB after pretreatment and fermentation. The data provide prospects of utilizing this IL in comparison to imidazolium based IL for pretreatment of biomass.

Keywords: *Bacillus aestuarii,* Ionic liquid, Sugarcane bagasse

Pharmacological effects of ficus carica leaves extract in animal model of amnesia

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Now a day's herbs have been widely used to cure many ailments because of their beneficial constituent. *Ficus* species; a huge tropical, deciduous, evergreen tree, are rich source of polyphenolic and flavonoids compounds which possess strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases such as neurodegenerative and hepatic diseases. Present study was designed to monitor pharmacological activities of *Ficus carica* Linn. *Ficus carica* leaves extract in healthy and memory impaired rat model. Thirty male Albino Wistar rats were divided into five groups: (1) Control (2) Memory impaired (3) Positive control (4) FCL treated healthy and (5) FCL treated memory impaired rats. FCL extract was given at dose of 250 mg/kg daily for 3 weeks. Scopolamine at a dose of 0.5 mg/kg was injected before evaluation of behavior. After three weeks behavioral (memory, anxiety, depression), biochemical (Hepatic and cardiac markers and antioxidant enzymes) and neurochemical (acetylcholine) parameters were studied. Results showed that FCL improved recognition and long term memory by enhancing levels of acetylcholine and antioxidant enzymes. FCL also exhibited anxiolytic and anti-depressant effects in healthy rats. FCL treatment reversed the scopolamine-induced anxiety and impairment in memory. These results may help to improve therapeutics in oxidative stress induced disorders.

Keywords: *Ficus carica* leaves (FCL), oxidative stress, memory, anxiety, antioxidant, neuroprotective

Interspecific Relationship within Genus *Abutilon*: Coding and Non-coding regions of Chloroplast DNA markers

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Abutilon is complex genus in the family Malvaceae. On the basis of morphological data, it is taxonomically complicated genus, therefore, there is a need to resolve ambiguities and find out interspecific relationships using molecular data. Aim of the current study is to inference of interspecific relationship within genus *Abutilon*.

DNA were extracted from selected plant species, two regions of Chloroplast DNA; coding *(mat*K*)* and noncoding intergenic spacer *(trn*L-*trn*F*)* were amplified, purified then sequenced. Obtained sequences were analyzed and submitted to GenBank. The phylogenetic analysis for inference of species relationship was carried out using Neighbor Joining (NJ) method through MEGA software and maximum likelihood (ML) analysis.

The result revealed that the noncoding region *(trnL-trnF)* of the chloroplast DNA worked comparatively well in resolving association between investigated species as compare to coding region *(matK).* This study Inferred the sister association of the *Abutilon indicum* with *Abutilon bidentatum, Abutilon pannosum* with *Abutilon fruticosum*. Furthermore, *Abutilon theopharasti* was found sister to above mentioned species.

Results provide valuable preliminary data for expressing and more analyzing taxonomic demarcation of *Abutilon* species from Pakistan. Increased sample size and use nuclear and mitochondrion DNA regions may provide better resolution between species.

Clinical confidence and predictability in aligner therapy technology (Invisalign Clear Aligners) by Digital Orthodontics

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Abstract: Invisalign is an orthodontic technique that uses a series of computer-generated custom plastic aligners to guide the teeth gradually into proper alignment. aligners fabricated from digitally manipulated models are the largest growing area in aligner treatment. This growth is most likely attributable to the ability of these aligners to be used to treat malocclusions ranging from minor to complex, including both posterior and anterior teeth. Using digital technology to control tooth movement, intricate and precise tooth movements can be staged for each sequential aligner.

Methods: The advent of intraoral scanners has made the process of digitization simpler and has eliminated the need for a third party to convert an impression into a virtual model. An instant virtual model from an intraoral scanner, in combination with multiple software platforms (Clincheck), allows the orthodontist to manipulate teeth with or without the assistance of a technician. That, along with a three-dimensional printer, allows the orthodontist to make aligners easily.

Result: The unique combination of computerized virtual treatment planning and the first of its kind use of stereolithographic rapid prototyping technology for mass custom manufacturing gave Invisalign a commanding leadership role in aligner therapy.

Conclusion: Successful treatment of many malocclusions with proper tip, torque, arch form, and aesthetic crown inclination is possible to achieve with clear aligners. Align Technology provides the most advanced clear aligner technology in the marketplace to date. Invisalign-branded clear aligners, using the proprietary plastic material and innovative SmartForce enhancements, provide predictable high-quality outcomes in the hands of a competently trained orthodontist.

In-silico based inhibition of hiv-1 subtypes by a pholiota squarrosa lectin

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HIV-1 envelope spike consists of GP120 and GP41. GP120 is the site of interaction with the host cell. GP120 protein of HIV-1 is masked with N—glycans, also known as "glycan shield". N-glycosylation of glycoprotein (GP120) is an important event in HIV-1 infection. The N- glycans affect cell-virus attachment and identification of GP120 by immune system. The blockage of N-glycosylation of HIV-1 glycoprotein can aid in developing therapeutic strategies against HIV-1. The study of N-glycosylation has become an area of extensive research in current areas of research in virus biology. Lectins are carbohydrate binding agents. The lectins that can target N-glycans on the surface of HIV-1 GP120 are potential antiviral agents. The lectin of our interest is a novel Pholiota squarrosa lectin (PhoSL), isolated from mushroom *Pholiota squarrosa*, that identify N-glycans having α1,6-linked fucose residues. In HIV-1 glycoprotein, fucose was found associated only with the innermost GLcNAc of N-linked glycans. In this study, the main objective is to observe the possible interactions among Pholiota squarrosa Lectin and GP120 glycans of HIV-1, by using in-silico based methods. Molecular docking of GP120 of HIV-1 subtypes and the Pholiota squarrosa lectin (PhoSL) was performed by using Patch Dock and Swiss-Model. Interactions between PhoSL and HIV-1 GP120 were analyzed by PLIP (Protein-Ligand Interaction Profiler) server. The molecular docking results showed that the Pholiota squarrosa lectin (PhoSL) can form metal complexes along with non-covalent interactions with GP120 of HIV-1. It can be concluded that the Pholiota squarrosa lectin may serve as a potential agent to block N-glycosylation of HIV-1 glycoprotein. However, empirical data is required to confirm its antiviral potential.

Keywords: HIV-1; N-glycans; GP120; lectin

Genetic cause of type 2 diabetes by identification of TCF7L2 gene polymorphism association

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Abstract: Transcription factor 7 like 2 (TCF7L2) is one of the type 2 diabetes susceptible gene though, data on the genetic cause of type 2 diabetes in South Asian countries are very scarce. The objective of this study was to assess the association between the TCF7L2 gene and type 2 diabetes. A case control study was conducted on blood samples from 100 controls and 100 type 2 diabetic patients. DNA extraction and genotyping was done in all recruited subjects. Alleles were visualized by horizontal agarose gel electrophoresis. Data was analyzed by IBM SPSS version 20. Results revealed significant association between TCF7L2 polymorphisms and T2D with p-value less than 0.05. This study replicates the presence of association between TCF7L2 variants and T2D as observed in other ethnicities. Furthermore, studies in the same populations are required to support these findings so that more definitive evidences can be drawn.

Biofilm forming potential of clinical MRSA isolates from Lahore, Pakistan.

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Abstract: Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the causative agents among life threating infections. The emerging antibiotic resistance has further worsened the situation. This has led to few antibiotic choices of the last resorts drugs like vancomycin for empiric therapy whose efficiency is questioned in case of biofilm forming strains. The current study aimed to find the biofilm forming potential of MRSA isolates along with their antibiotic resistance profile. *S. aureus* clinical isolates were screened by biochemical tests for MRSA and 150 MRSA isolates were identified phenotypically by cefoxitin antibiotic disc (FOX-30) according to Clinical and Laboratory Standard Institute (CLSI) 2019 guidelines. All the MRSA were confirmed genotypically by *mec*A gene and *S. aureus* specific *nuc* gene. Biofilm formation was assessed by congo red agar (CRA) assay and slime layer quantification method. By CRA method, strong biofilm formation was observed in 18.18% while 21.82, 40 and 20% were medium, weak and non-biofilm formers, respectively. Slime layer detected 29.09, 50.91, 8 and 2% of the isolates as strong, moderate, weak and non-biofilm formers, respectively. Biofilm forming genes *ica*A and *ica*D were detected in 51.71 and 83.70% of the MRSA isolates. Antibiotic resistance was higher in biofilm formers and weak biofilm formers were completely susceptible to linezolid, amikacin and trimethoprim/sulfamethoxazole. In conclusion, biofilm forming potential in clinical MRSA strains is very high and routine antibiotic sensitivity in clinical settings may lead to failure of empiric antibiotic therapy.

Keywords: Biofilm, MRSA, *mec*A, *ica*A, *ica*D.

Fluconazole resistance among *Candida* species: An obstacle in the treatment of Candidiasis

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Candidiasis, the most prevalent opportunistic fungal infections caused by Candida species are usually associated with high morbidity and mortality rates. *C.albicans* was considered as the only Candida specie responsible for causing this disease but recently non *albicans Candida* species (NAC) are emerging as new pathogens to cause this infection. This change in epidemiology of Candidiasis is also responsible for aggravation of disease as these NAC species are more resistant to antifungals as compared to *C.albicans*. This study was conducted to evaluate the prevalence of *Candida* species causing Candidiasis in Karachi City as well as their susceptibilities to commonly used antifungal drug Fluconazole. For this purpose, 562 isolates of Candida were obtained from diagnostic laboratory from known cases of Candidiasis and were identified by using conventional methods such as microscopy, germ tube test, growth characteristics on Chromogenic media, Corn meal agar and Carbohydrates fermentation and assimilation. The doubtful species were identified by using Remel RapID™ yeast plus kit. The results were statistically analyzed by SPSS 16.0 version software. The result revealed emergence of non-*albicans Candida* species (45.5%) in appreciable number along with *C.albicans* (54.5%). Among NAC species, *C.tropicalis*, *C. glabrata*, *Candida rugosa*, *C.krusei* were isolated predominantly. These isolates were screened for their susceptibilities towards Fluconazole by using disc diffusion technique. Fluconazaole, which is drug of choice in candidiasis because of its efficacy, less toxicity, cost effectivity and oral administration, demonstrated higher number of resistant isolates of NAC as compared to *C.albicans*. Overall 10% of all the Candida isolates were found resistant to fluconazole while specie wise resistance profile revealed highest rates from *C.tropicalis* (12%), *C.galabrata* (13%) and *C.rugosa* (13%) as compared to *C. albicans* (9%). The high fluconazole resistance rates observed among NAC species depict an upcoming threat for public health and therefore continued surveillance is required for proper management of Candidiasis.

Key Words: Fluconazole, Candidiasis, *C.albicans*, Non-*albicans Candida*

A novel drug combination therapy for epilepsy increasing the latency to continuous seizures and survival rates in pilocarpine induced epilepsy mice model

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Epilepsy is an excitatory neuronal disorder that effects 1% of the worldwide population. There are several anti-epileptic drugs available for its treatment but approximately 35-40% of the patients get resistant to them. This requires establishing new therapies for the resistant patients for which the exact cause should be identified. Recent studies have shown that inflammation have a role in the pathophysiology of epilepsy and proves it through the identification of inflammatory markers in human patients and in animal model. Therefore, the purpose of this research is to examine the impact of levetiracetam (LEV) and diclofenac sodium (DFS) combination on epilepsy and to analyze their effect on inflammatory marker expression in pilocarpine induced epilepsy mice models. In different groups of NMRI mice, LEV alone and in combination with DFS were given for 3 days. On the 3rd day after administering the required drugs pilocarpine challenge was given, intraperitonially. Behavioral changes were observed for 90 mins after the administration of pilocarpine; for the latency of first seizure, continuous seizures, duration of continuous seizures and mortality. Upon observations the combination of LEV and DFS showed increase in the latency of continuous seizures and survival time. The result of this study shows that DFS enhances the efficacy of LEV and can be given in combination with LEV for epilepsy treatment after further investigations. Brain samples are stored for further future investigations regarding changes in expression of inflammatory markers.

Identification of small molecular Inhibitors of HslV protease; Towards New Antimicrobials

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HslVU is an ATP-dependent protease which is involved in the removal of unwanted and misfolded proteins from the cell. HslVU is considered as the ancestor of 26S proteasome, which is composed of 20S protease and the 19S regulatory caps. HslVU consists of two heat-shock proteins; HslV protease and HslU ATPase/chaperone. HslV dodecamer is flanked on both sides by HslU hexamers. Both 26S proteasome and HslVU share close structural similarity. During the present studies, homology modeling of *E.coli* HslV monomer complexed with NLVS was performed using MODELLER software followed by protein-ligand docking studies using AutoDock Vina. Sixty Four non-peptidic molecules related to eleven different chemical scaffolds were screened. The derivatives of triazole, coumarin, thiosemicarbazide, thiazole and indole families showed good docking scores and compatible interactions within the HslV active site. Here, we believe that these small non-peptidic molecules can compete with the natural substrates in binding with HslV protease inside the cell and can hinder the protein degradation phenomenon required for cell survival. The inhibition of HslV protease will lead to the accumulation of misfolded and unwanted proteins, ultimately leading to the cell death. The present research has opened a new door for treating an array of microbial diseases.

Efficacy of Antibiotics with Efflux Pump Blockers on Multi-Drug Resistant Bacterial Strains

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Abstract:
The mechanism of multi drug resistant in many microbes has led to the prevalence of several life-threatening diseases such as Tuberculosis caused by *Mycobacterium tuberculosis*, skin and other structure infections caused by Methicillin Resistant *Staphylococcus aureus*, Candidiasis caused by *Candida albicans,* and many other pathogenic infections. To overcome the resistivity to drugs, new antibiotics with better efficacy and reduced resistivity are being devised. Older antibiotics are being tested again to get efficacy at higher doses. Along with all these drug discovery, development and trial studies, the focus of interest is the development of Efflux Pump Blockers. All living organisms have the efflux pumps in their cellular structures to remove waste material and unwanted substances from the cells but the efflux pumps have also been known to flush out the administered antibiotics from the cell as well. This has led to the biggest problem of resistivity in microbes as they remove the antibiotic, and the microbe persists for a longer period causing diseases to severe extent. This resistivity can be controlled by using molecules which block the efflux pumps so that antibiotics stay within the cell and do their required function effectively. The objective of the research was to study the effect of efflux pump blockers when used in combination with different types of antibiotics. The Efflux Pump Inhibitors used were Tamoxifen and Verapamil. The antibiotics included in the study were Chloramphenicol and Nalidixic Acid. They all were used against five strains of Methicillin Resistant *Staphylococcus aureus*. It was found that when the antibiotics and efflux pump blockers were tested solely against the bacterial strains, high resistivity was observed. When the antibiotics were used in combination with the efflux pump blockers, the results were surprisingly very effective. The growth of bacteria was significantly inhibited, and efficacy of antibiotics was increased probably by preventing their removal from the cells by blocking the efflux pumps. Thus, research should be more focused to study about the efflux pump blockers as they can serve as useful agents in treatment of pathogenic diseases.

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Keywords:

Efflux Pump Inhibitors, Antibiotics, Methicillin Resistant *Staphylococcus aureus*, Minimum Inhibitory Concentration (MIC) Test, Minimum Bactericidal Concentration (MBC) Test

Role of USP2 Inhibitor In Cell Cycle Arrest Via Degradation of Cyclin D1 In Triple Negative Breast Cancer

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ABSTRACT:

Breast cancer is the most significant and second cause of death in women throughout the world. Among Asian countries, Pakistan has the highest incident rate with late prognosis and absence of early detection markers that increase overall burden of breast cancer. Triple negative breast cancer (TNBC) is an aggressive type of breast cancer that does not respond to major targeted chemo and hormonal therapy. TNBC with high rate of relapse and metastasis accounts approximately 17.28% population in Pakistan. Treatment regime embraces neoadjuvant therapy and radiotherapy with pre or post-surgical processes depends upon tumor stage. Aberration in various cellular signaling pathways causes cell proliferation and growth. These pathways are regulated by receptors which are further controlled by various post translational modifications (PTMs). Ubiquitination is a kind of PTM that comprises of complex cascade which play crucial role in stability and homeostasis of different cellular protein. Ubiquitin specific protease 2 (USP2) possess oncogenic properties controls the fortune of several oncogene substrates by restrains proteasomal degradation. USP2 has a pivotal role in Fatty acid synthase (FAS) metabolism and its overexpression prevents FAS degradation as a consequence it inhibits apoptosis in cancer. Our study interrogates an anti-cancer role of ML364, USP2 inhibitor in TNBC. We demonstrated cytotoxicity of ML364 and identified differential expression of genes upon treatment with USP2 inhibitor through real time quantification at molecular level. In present study we also investigate expression profile of USP2 in tissue sample of BC patients .USP2 inhibitor might be beneficial for the treatment of TNBC and could be used as potential anticancer agent.

Keywords: Breast cancer, Ubiquitination, USP2, ML364.

A COMPARATIVE CHARACTERIZATION OF KERATINASE ENZYMES FROM INDIGENOUS FUNGAL AND BACTERIAL ISOLATES

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ABSTRACT

Standard microbiological technique was used for the isolation of keratinolytic microorganisms from soil samples. Primary screening for proteolytic activity of isolates was performed by using skim milk agar. The keratinolytic activity was observed by submerged fermentation using chicken feathers as substrate. The enzyme activity was spectrophotometrically measured by using commercial keratin azure as keratin substrate. The isolated keratinolytic microorganisms were comprised of *Chrysosporium asperatum*, *Chrysosporium keratinophilum*, *Entomophthora coronata*, *Absidia* sp. and *Bacillus subtilis*. From these isolates *C. keratinophilum* and *B. subtilis* produced largest zone of hydrolysis on skim milk agar among all the isolated species. The crude keratinase obtained by submerged fermentation was characterized and it was revealed that optimum time, temperature and pH for the production of keratinase by *C. keratinophilum* was 7 days, 30^0C and 9.0 respectively, whereas, optimum time, temperature and pH for the production of keratinase by *B. subtilis* was 3 days, 37^0C and 7.0 respectively. The enzyme activity of *C. keratinophilum* and *B. subtilis* was determined to be 220U/ml and 260U/ml respectively.

In-silico Discovery of Non Peptidic Activators of Bacterial HslV Protease

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HslVU is mainly responsible for the elimination of aberrant polypeptides by catalyzing energy- dependent proteolysis in prokaryotes. HslVU protease-chaperone complex is composed of two components i.e. HslV protease and HslU ATPase. The functional HslVU complex forms when hexameric HslU binds at both ends of HslV dodecamer while the HslU carboxy terminal peptide or C-tail is distended and intercalated into cleft between adjacent HslV subunits. This intercalation of HslU C-tail causes a conformation change in the HslV active site which accelerates the proteolytic activity of HslV. HslVU is a novel drug target for antimicrobial research due to its presence in pathogenic microbes and simultaneous absence from human beings. Due to extensive universal occurrence of microbial resistant to antibiotics, there is an emerging need for identifying new molecules with antimicrobial properties. Using in-silico techniques, we have identified small non-peptidic molecules capable of activating HslV protease by binding with HslU C-tail-binding pocket. The identified lead molecules are derivatives of acridine, pyridine, indole and chromone compound families. Pro-tein-ligand docking results revealed that their binding energies are much higher than C-tail and involved in making hydrogen bonds and hydrophobic interactions with important residues of HslU C-tail-binding pocket. The interaction of these chemical molecules with HslV protease will abnormally increase the intracellular protein degradation and possibly cause death of potential microbes without affecting human beings.

Drug Repurposing-Mining the Haystack for Cancer and Bone Fragility

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ABSTRACT:

Drug repurposing appears as an efficient approach for drug design and development. Increasing burden of breast cancer and associated consequences demands for the urgent development of newer and safer therapeutic strategies.

Occurrence of cancer is higher in patients with the history of using anti-depressants. Both clinical and experimental studies have demonstrated that stress may influence tumor progression. Anti-proliferative activity of certain antidepressants are evidenced from the literature. Selective serotonin reuptake inhibitors (SSRIs) are used to treat hypertension in cancer patients. Bone cells offer an enriched soil for growth of cancer cells, and bone fragility is the rising consequence of breast cancer. We therefore, aimed to analyze the influence of commonly used SSRIs, fluoxetine, and paroxetine, sertraline hydrochloride along with other commonly used antidepressant amlodipine besylate in a panel of breast cancer cell lines. Sertraline hydrochloride was selected for further evaluation of possible mechanism underlying its pro-apoptotic effect. To the best of our knowledge no study has been carried out to evaluate the anticancer potential of sertraline hydrochloride on AU565 cell line.

Through systematic evaluation of around 300 commercially available drugs and small molecules, we have identified megestrol acetate, pregnenolone, econazole nitrate, enalaprilat dihydrate as potential agents for osteoblast proliferation and differentiation.

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Fungal contamination in Smokeless tobacco products and their possible consequences on human health

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Smokeless tobacco is related with a variety of oral and systemic diseases precisely with oral and pharyngeal cancers.Variety of smokeless tobacco products (STP) are available and consumed in Pakistan including naswar, mainpuri, gutkha, mawa, khiwam etc. These products have ample amount of tobacco as its main ingredient but there are no health warnings on packaging resulting in many individuals failing to realize the life-threatening consequences of consuming these addictive products. Present study was focused on the evaluation of fungal contamination in these products. Moreover, the oral microbiological assessment of consumers using these products was also carried out in this study. A total of 600 different samples of smokeless tobacco products were collected from different parts of country including Karachi, Lahore, Peshawar ,Quetta and Azad Kashmir. These products were processed for the isolation of fungi by spread plate method. The isolated fungi were identified conventionally by their macroscopic and microscopic characteristics. *Aspergillus fumigatus* and *Aspergillus flavus* were revealed as the most dominant species from these products while Naswar was observed as the most contaminated product among all smokeless tobacco products. . Furthermore, the oral swabs from consumers of these products were also collected and streaked on fungal media for the isolation and identification of fungi. *Aspergillus nidulans,Aspergillus niger* and *Aspergillus tererus* were predominantly isolated from these swabs. The established evidence of occurrence of pathogenic fungi in the most consumed STPs pose a great danger on human health as it is consumed by majority of individuals irrespective of age and class. The results of the study conclude an alarming situation and needs swift management and awareness among tobacco consumers. Prompt measures must be adopted by health authorities to safeguard the health of the general public, especially the upcoming youth.

Key words: Smokeless tobacco products, Nicotine, fungal contamination, Gutka

Purification Strategies and Biochemical properties of a Highly Catalytic Endo 1,4-β Xylanase from *Aspergillus niger* KIBGE-IB36

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Abstract

Xylanase degrades xylan by randomly hydrolyzing the β-1,4-glycosidic bonds to produce xylo-oligosaccharides. Xylanases have gained considerable attention because of their tremendous applications in industries including animal feeding, food baking and pulp bleaching. Previously *Aspergillus niger* KIBGE-IB36 produced xylanase under submerged fermentation which degrades the complex hemicelluloses containing xylan. The purpose of current study was to purify xylanase and analyze its biochemical properties. Enzyme properties were evaluated following chromatographic purification. It was also observed that the purified enzyme showed stability with various metal ions, organic solvents and detergents. The hydrolysis pattern of purified xylanase showed efficient xylan degrading pattern and produced xylose as an end-product. The relative amino acid composition showed greater amount of lysine and N-terminal sequence of purified xylanase revealed that the enzyme has N-glycosylation properties that make the xylanase more stable and have high catalytic potential. Hence, the results obtained from the current study showed that the purified enzyme from *Aspergillus niger* KIBGE-IB36 have potential to be used in different industrial applications mainly in animal feed, paper and pulp industry and enzymatic pre-treatment of agro-industrial wastes.

Keywords: Xylanase, Enzymology characteristics, Catalytic efficiency, Xylo-oligosaccharides, N-glycosylation

Ubiquitin Specific Protease Mediated Regulation of c-MYC in Diffuse Large B Cell Lymphoma

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Diffuse Large B-cell Lymphoma (DLBCL) is morphologically characterized by effacement of lymph node architecture and diffuse growth of neoplastic large B lymphoid cells with enlarged nuclei. The disease accounts for 40% of all Non-Hodgkin's Lymphoma cases in Pakistan. Like other cancers it's also underpinned by dys-regulation of c-myc. The primary objective of the present study is to explore the responsible USPs that maintain cellular turnover of c-Myc in DLBCL. In total, 100 histologically diagnosed cases of DLBCL were inducted in the study. The tissue was subjected to immunohistochemical examination for c-Myc and it's known (other cancers) regulators namely USP28, USP36 and USP37. The expression was quantified using IHC profile module integrated with Image-J. Three thresholds of signals were measured i.e. low positive, positive and high positive. The data then statistically analyzed using Graph Pad Prism. Correlation of expression of c-Myc against USP28, USP36 and USP37 shows no statistically significant correlation for the expression of these proteins with p-value ranging from (0.247 to 0.560) using low positive signals. Under positive signals, USP36 expression was found to be positively co-related (p=0.04) with c-Myc expression. Interestingly, at high positive signals, USP37 expression was found positively co-related (p=0.001) suggesting USP37 is the major regulator of c-Myc in DLBCL. In summary, the data points that regulation and/or stabilization of c-Myc in DLBCL is mainly the function of USP37 and in part USP36. The findings may further be exploited in developing inhibitor against c-Myc-USP37 or c-Myc-USP36 molecular interactions.

The study is supported by Higher Education Commission, Government of Pakistan.

Key words: *DLBCL, USP28, USP36, USP37 , c-MYC*

Structural Phylogenetics of Hepatitis B Virus Oncogene, HBx

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Hepatitis B Virus (HBV) is one of the leading causes of viral associated Hepatocellular Carcinoma. To date one oncogene, HBx is known for the virus encoding a 154 amino acid long protein. In this study we have modelled full length molecular structure of the HBx by employing strategies of iterative threading approach followed by thermodynamic and structural refinements. In addition phosphorylation potential of the protein was also explore on the basis of both primary and tertiary structures. The findings were then aligned with the orthologous sequences of HBx of different HBV genotypes and HBV of other primates and rodents. Sequence alignment showed that p53 binding regions are highly conserved amongst orthologous sequences compared to regulatory domain of HBx. Xtal pred analyses predicted low probability of crystallization of HBx thereby difficult to be structurally resolved empirically. The modelled structure showed extensive intrinsically disordered region at the N-terminal of the protein whereas the C-terminal is alpha helical in shape. Structural comparison of the full length model of HBx of different HBV genotypes of humans and other animals showed subtle variation in the Cα back bone architecture where RMSD was found to be from 0.04Å to 0.68Å. Ser 25, Ser41 and Ser43 showed significant potential of being phosphorylated amongst different HBx orthologues. Consistently the most commonly kinase predicted to undertake HBx phosphorylation was found to be Unsp. In summary, the findings will provide insights to the evolution of HBx and its neo-functionalization that leads to the evolution of its oncogenic attributes.

Keywords: Hepatitis B Virus, Hepatocellular Carcinoma, Viral Evolution, Phosphorylation, Oncogene

Investigating NGF and Partner Proteins in Irreversible Pulpitis

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Irreversible Pulpitis is characterized by the inflammation of the dental pulp. It is manifested as spontaneous and lingering pain in teeth that does not subside even after removal of the evoking stimulus. It accounts for 19.4 % of total dental diseases in Karachi. The investigation aims to explore and compare expression of genes involved in NGF and BDNF pathways in healthy and irreversibly inflamed dental pulp samples. Briefly, 40 subjects were recruited for each of the two groups, namely control (healthy/normal) and test (subjects with irreversible pulpitis). Pulp was extirpated from the teeth and preserved as per standard protocols. All tissue samples were subjected to histological and immunohistochemical examination for the expression of NGF, BDNF, NT-4/5, TrkA, TrkB, Nedd4-2 and USP-36 genes. Signals for expression were quantified under three thresholds that are high positive, positive and low positive using IHC profiler module integrated with ImageJ software. Depending on the data, statistical analysis was done by GraphPad Prism. Statistically significant differences were found in vessel diameters (p-value 0.0072) and nuclear diameters (p-value 0.0099) when compared between test and control groups. Where, drops were noticed in both histologic parameters in irreversible pulpitis compared to control. Several assessed genes including: NGF, BDNF, NT-4/5, Nedd4-2 and USP-36 genes were found over expressed in test compared to control group. In addition, positive correlations were found between expression of NGF with BDNF (p-value <0.0001), Nedd4-2 (p-value 0.0003) and USP36 (p-value <0.0001) and BDNF with NT-4/5 (p-value 0.0001), Nedd4-2 (p-value <0.0001) In total, our findings are the first to provide a more resolved and composite picture of the molecular interplay of Nerve Growth Factor that underpins irreversible pulpitis.

Key words: Irreversible Pulpitis, NGF, BDNF, USP-36, Nedd4-2

Optimization of High Resolution Melting (HRM) Technique for *MTHFR* C677T Polymorphism in Cardiac Malformations

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High Resolution Melting Analysis is relatively new PCR based genotyping technique, which investigate the data of known SNPs. This new mutational analyzing technique is time saving and cost effective. The basic concept behind it to provide data points using saturated dyes which are usually DNA intercalating dyes and measures the fluorescence through software. HRM technique needs a real time PCR with its software. The reaction is designed using SNP classifications; Class I to IV. It is recommended for short amplicons, nearly 50 to 200 bp in length for more specificity and uniqueness. The closed system based analysis requires super-mix containing master-mix and a saturating dye (Eva-Green). The real time PCR was conducted using the manufacturer's recipe (Biorad). The peaks were investigated by comparing known and unknown samples. The best results was obtained using 50 µg/ul DNA. Congenital heart defect patients were recruited from NICVD for the study. Positive and negative controls were used for verification. For optimization, the effect of two-step or three step protocols both were tried. As this is a GC rich, three-step analysis give good results. During optimization the role of 2.5 mM $MgCl_2$ showed its effectiveness in amplification and providing differentiated data points. Data was collected between temperature ranges 65ºC to 95ºC in 0.5ºC to 0.2ºC increments and cooling effect from 60 to 40ºC. The narrower increment gives the better melt curve. The aim of the study is to optimize HRM protocol for known SNP(s) to set up the diagnostic parameters. This technique might also be useful for unknown genotypes and used as a diagnostic tool. This study was the initial step using most studied gene *MTHFR*.

Keywords:

High resolution melt analysis, melt curve, *MTHFR*, cardiac malformations

Birth and Death of *CYLD* Gene Paralogues in Vertebrate Lineage

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CYLD, cylindromatosis, gene encodes an ubiquitin specific proteases that stabilize protein linked with an array of biological role, NFkB mediated inflammation, p53 and RIG mediated apoptosis. The present investigation aims to explore the evolution of *CYLD* gene in vertebrate lineage underlying genomic mechanism. Briefly, genomes of species of vertebrate lineages were explored for the presence of *CYLD* gene homologues. The retrieved sequences were aligned for C19 domain and phylogenetic tree was reconstructed with 1000 bootstrap replicates employing JTT+G evolutionary model. Phylogenetic tree was then realigned with genomic physical map of genes, gene structure and protein domain architecture. Finally, full length molecular models of ancestral form of CYLD, CYLDA and CYLDB were constructed and compared for structural attributes. Genome mining demonstrates variable number of homologues in different species of vertebrates with mammals have only one CYLD homologue whereas fishes have maximum of six homologues of respective gene. Phylogenetic analysis divided all sequences into two superclades, CYLD I and CYLD II. CYLD I comprises three major clades, CYLDA, CYLDE and CYLDC. Whereas, CYLD II coalesced two clades, CYLDB and CYLDD. Distribution of species amongst clades and superclades points that first two rounds of whole genome duplication at the base of vertebrate led to the origin of four CYLD paralogues, CYLDA, CYLDB, CYLDD and CYLDE. In comparison CYLDC, CYLDF and CYLDG are the product of third and fourth round of whole genome duplication in fish lineages. Primary and tertiary structural comparison of paralogous proteins and site specific selection pressure points to the neofunctionalization and/or sub functionalization among different CYLD paralogues. Findings of the present study are the first to resolve the evolutionary roadmap of CYLD at genomic resolution. Thereby covering the lacuna in the understanding of CYLD biology.

The study is supported by the Higher Education Commission

Keywords : CYLD, USPs, Cylindromatosis, phylogenomics, whole genome duplication.

A comprehensive bioinformatics analysis and Machine learning model for *Mycobacterium Tuberculosis*

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The progression in the development of next-generation sequencing (NGS) technologies has been revolutionized due to the decrease in the sequencing cost and latest bench-top availability of the sequencing NGS instrument. As a result, it has increased the chances of whole-genome sequencing (WGS) technology to be used for bacterial strain characterization and identification as a routine tool in laboratory testing (Walker et al., 2017). This advancement has significantly prompted the epidemiological reconnaissance of substantial pathogens, for example, the Mycobacterium tuberculosis (MTB) (Dheda et al., 2017; Merker et al., 2017; Walker et al., 2018; Zignol et al., 2018). In 2017, TB remains one of the major concern and one of the 10 leading causes of death worldwide, with 10 million new cases due to the constant rise of multidrug resistant MTB strains (MDR -TB) (WHO, 2018). This project aims for the development of a variant calling pipeline for targeted genes to characterize genome variation in MTB by determine SNPs associated with drug resistance in four first line anti-tuberculosis drugs that includes Isoniazid (INH), Rifampicin (RIF), Ethambutol (EMB) and Streptomycin (STR). As well it will also develop a deep leaning resistance model for genes that makes the MTB strain resistant against EMB anti-TB drug. An automated pipeline for MTB NGS data analysis along with a gene resistance prediction model will help clinical reporting of tuberculosis and its resistance against the drugs for better prognosis and treatments.

Effect of lycopene supplementation on liver enzymes and antioxidant enzymes in thioacetamide induced liver cirrhosis: study in rats

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Lycopene ($C_{40}H_{56}$) is an aliphatic hydrocarbon, one of the 600 known naturally occurring carotenoids, the most abundant carotenoids found in tomatoes, tomato products and other red fruits. Lycopene powerful anti-oxidative role is associated with its ability to perform as a free radical scavengers against reactive oxygen species (ROS). Lycopene also worked as an anti-inflammatory response, reduces chances of cancer, prevents lipid peroxidation, balances normal cell metabolism, and slow down oxidative damage. More dietary consumption of tomato products can decrease LDL cholesterol levels and enhances HDL cholesterol levels. Liver cirrhosis is the alteration of the liver cells that initially produces by the high accumulation of collagen in the liver tissues. The purpose of the current study was to study the protective effect of lycopene in thioacetamide induced liver cirrhosis. For this study twenty four male Albino Wistar rats were randomized into four groups each group was consisted of six rats. Group I: control healthy group rats; Group II: received thioacetamide; Group III: received thioacetamide and tomato extract (lycopene) and; Group IV: received tomato extract orally for 12 weeks. Biochemical estimations includes estimation of antioxidant enzymes, liver enzymes, lipid profile test and glucose levels. Markedly enhanced concentration of bilirubin, ALT, ALP, catalase, MDA, cholesterol, triglyceride and LDL were observed in cirrhotic rats and decreased concentration of SOD enzyme and HDL level were observed in thioacetamide treated rats. Tomato extract (lycopene) supplementation decreased the concentration of MDA, Catalase, Bilirubin, ALT,ALP, cholesterol, triglyceride and LDL in Thioacetamide and lycopene treated and alone Lycopene treated groups. While increased concentration of SOD and HDL were observed in thioacetamide and lycopene treated and alone Lycopene treated groups. These results attribute that lycopene attenuates liver cirrhosis.

Keywords: Liver cirrhosis, Lycopene, Thioacetamide, Liver enzymes, Antioxidant enzymes.

Age-related changes in the functional status of mitochondria and the effect of natural compounds on it

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According to available data, there is a decrease in the functional parameters of mitochondria with the appearance of an active form of oxygen, decreased activity of respiratory chain enzymes and increased sensitivity of mitochondria and cells to apoptotic signals. To prevent premature aging and increase longevity, created special geriatric preparations, which affect the structure, function and metabolism of an aging organism. In recent years, herbal remedies have been used, including natural flavonoids, diterpenoids and polyphenols.

In our research work, we investigated the effect of glycyrrhizic acid polyphenolic and flavanoid complexes, isolated from the plant (Glycyrrhiza glabra) on respiratory enzymes, oxygen utilization, and ATP synthesis in mitochondria. In study used differential centrifugation, polarographic, spectrometric, other modern biochemical, and biophysical methods. According to the study, was detected the activity of enzymes in the mitochondria increases with age, a decrease in protein biosynthesis and respiratory control. The effect of glycyrrhizic acid-based polyphenols on the biochemical parameters of respiratory enzymes, oxygen utilization, and ATP synthesis in mitochondria was studied. Under the influence of complex compounds, it was found that the active enzymes of the respiratory chain are restored, and respiratory control is normalized.

In the future, on the basis of these complex compounds, it may be possible to create and develop medicinal geriatric preparations for production based on local raw materials.

Key words: mitochondria, geriatric drugs, respiratory enzymes.

DETERMINING THE LEVEL OF HISTAMINE IN THE EXPERIMENTAL ANAPHYLACTIC SHOCK

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It is known that an allergic reaction is accompanied by activation mast cells of tissue sand basophiles of blood and releasing them whole group of mediators in the pathochemical stage, among which certain important role is played by histamine. The presence of receptors for histamine on a variety of cells involved in immune response and a wide range of effects mediated by this mediator - the facts that gave every reason to be regarded as a potent histamine, an endogenous immune modulator. Regulatory activity of mediator on cellular and humoral immunity, including products of reaginsis mediated by T-lymphocytes with receptors to it. Interaction of histamine with receptors on the cell surface activates the adenylcyclase and it is followed by increasing of concentrations of cyclic AMP, which leads, in particular, to inhibition of the ability. Given the important role of histamine in the pathogenesis of allergic reactions, for choosing the right antihistamines and anti-allergy drugs, we set a next goal: to determine the level of histamine the blood and lymph in experimental anaphylactic shock.

Materials and methods. Experiments are carried out in two series: In I series of experiences these data are defined at 9 rabbits, reproduced with anaphylactic shock. As a control were studied parameters of histamine in the blood and lymph of intact rabbits. For reproduction of an acute anaphylactic shock the animals were sensitized by subcutaneous injection of 0.1 ml of horse serum resolution dose volume of 1 ml was introduced into a heart cavity. For the experience is required blood taken from the marginal vein of the rabbit, and lymph from the thoracic duct by the method of A. Kornienko modification on M. H. Aliyev and V.M.Mamedov.

The histamine level was determined by fluorometric method, which is justified on getting the fluorophore with 0.1% orthophthaleinaldehyde with the machine, «Bian-130».

Results. The results of investigation have shown that level of histamine increases both at the anaphylactic shock periods. But the increase in anaphylactic shock periods were more pronounced than in the sensitization periods. Thus, a 7-day sensitization histamine levels increased 1.6 times compared to the intact animals, but in period anaphylactic shock histamine levels increased 3.8 times, was 1.89 mmol in blood. After 30 minutes of shock histamine levels decreased 2.7 times in comparison with the period of shock.After reproduced anaphylactic shock three animals have died and blood was taken only from 6 animals after 30 minutes of an exposition anaphylactic shock.

In the lymph these rates were lower in comparison with the blood. Thus, on the 7^{th} day of sensitization of anaphylactic shock the level of histamine in lymph compared to the intact animals increased 1,4 ($p < 0,001$) times, and during shock increased to 2.19 mmol / L in 3 3 times higher than in intact animals. After 30 minutes of shock the level of histamine is decreased in 1. 7 times in comparison with the intact animals.

Glucose-6-phosphate dehydrogenase deficiency with combination hereditary hemochromatosis

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In the literature there are concepts of frequent damage of the same populations by hemoglobinopathies and hemolytic anemia caused by deficiency of the enzyme glucose-6-phosphate dehydrogenase (G-6-PD).

This enzyme is a polypeptide chain consisting of 500 amino acids. There is pyroglutamic acid at the N-terminus of the polypeptide chain and glycine at the C-terminus. In erythrocytes with reduced enzyme activity, clearly changes were revealed. These cells have reduced ability to form NADFH and bind oxygen, reduced rate of methemoglobin reduction, and reduced resistance to various potential oxidants. It can be assumed that under conditions of limited formation of reduced NADF in erythrocytes of people with G-6-FD deficiency, various substances, including other substances, cause decrease of serum iron (SI) level. On the other hand, 12.3% of patients with hereditary hemochromatosis are identified among the population of the Azerbaijan Republic. In connection with the deficiency of G-6-FD and hereditary hemochromatosis (HH) in the Republic of Azerbaijan, combinations of these pathologies are possible in the examined populations. Each of these diseases is accompanied by iron exchange disorders. Therefore, the purpose of this work was to study the display of iron in G-6-FD deficiency patients in a combination of HH.

We examined the blood of 49 children with G-6-FD insufficiency (21 girls and 28 boys). The level of G-6-FD activity was from 0 to 0.9 unitg/Hb. The iron exchange parameters were determined in all 49 patients which have detected deficits. Iron content (IC) in a serum ranged from 25 μmol/L to 70 μmol/L (norm 13-80 μmol/L), and transferrin saturation factor (TSF) ranged from 56.7% to 87.1% (norm 16-56%). These parameters in children with G-6-FD, were little different from those in the control group. It can be concluded that the carrying of G-6-FD deficiency is mainly not caused by the disruption of iron exchange and therefore the level of IC and TSF is normal. However, when comparing the results of the study on iron in 7 children it was observed a high level of this data. In the study of the distribution of the HLA A and B loci antigens in 5 children and relatives was found an increase frequency of A_3, B_7 and B_{14} antigens. These 5 children were diagnosed with hereditary hemochromatosis with a combination of G-6-FD.

The results of the study of venous blood of patients with G-6-FD enzyme deficiency make it possible to conclude that in most cases the bearing of enzyme deficiency in children is not accompanied by iron accumulation, but in the presence of combined forms iron indices increase due to HH bearing.

The study of some cytokines in the blood of diabetic glomerulosclerosis patients

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Diabetic glomerulosclerosis is one of the most common complications of diabetes mellitus. Much research has been devoted to the study of moleculyar mechanisms of renal damage. Recently, attention has been paid to the study of cytokine regulation of immune homeostasis in renal pathology. Taking this into account, the purpose of this research is to explore the role of some cytokines in the pathogenesis of immune disorders in diabetic glomerulosclerosis.

The study used the blood serum of 45 diabetic patients and 14 healthy individuals who applied to the horse-clinical biochemistry laboratory of Azerbaijan Medical University. Diabetes patients are divided into 3 groups:

15 patients without renal pathology, 2) 15 patients with conservative stage
of renal failure, 3) 15 patients with terminal (dialisis) stage of renal failure.

The IL-6 (interleykine-6) and TNF-α (tumor necrosis factor -α) cytokines concentration in the blood serum of the investigated individuals was determined by the immunenzyme method. Approval diabetes mellitus diagnosis the concentration of glucose, glucohemoglobine, insuline and C-peptide concentration is analysied in the blood serum of individuals. To confirm renal failure in individuals the blood concentration of creatinine and urea is analysied.

The results show a significant increase in the surveyed indicators in the blood serum of diabetes patients. The blood serum levels of IL-6 increased by 3.9 times and the level of TNF-α increased by 2.5 times in conservative stage of renal failure patients relative to the control group. İn terminal (dialysise) stage of renal failure this parameteries increased 5.9 and 4.4 times respectively. In diabetes patients without renal damages these indicators has grown slightly (2 times and less). Results show that the amount of cytokines increases significantly in the blood according to renal failure levels of pain increase. This confirms the need for cytokines to study for early diagnosis of renal damage in patients with diabetes mellitus.

Differential Metabolic Proteins Expression in Human Autopsied Brain regions of Schizophrenics

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Schizophrenia is a devastating multifactorial neuropsychiatric disorder with unknown etiology, although diverse neuropathological evidence suggests disturbance in neurotransmission system, neuro-anatomical abnormalities and impaired synaptic connectivity. In this era, proteomic findings are in prime focus because they reflect complex gene and environment interactions and is increasingly appreciatedvalued in pathophysiological mechanism of a disease state.

The present study focused on the identification and elucidation of differentially expressed proteins which can be used as possible biomarkers for schizophrenia and as an indicator of disease-derived protein dysfunction. In this study, Ccomparative proteomic analysis of 9 human schizophrenic autopsied brain tissue (cortex hippocampus and substantia nigra) and their respective controls, was performed by using 2DE. The differentially regulated spots were then identified by LC-MS/MS and validated by western blotting. Our data revealed differential expression of 10 proteins involved in energy metabolism, in brain tissue (cortex, hippocampus, and substantia nigra) of schizophrenics as compared to the normal controls. Among the 6 proteins of substantia nigra, phosphoglycerate mutase 1, ATP synthase subunit d, mitochondrial and malate dehydrogenase cytoplasmic are downregulted, while glyceraldehyde-3-phosphate dehydrogenase, 4-trimethylaminobutyraldehyde dehydrogenase, and alcohol dehydrogenase are upregulated. In cortex, triosephosphate isomerase, electron transfer flavoprotein subunit beta, and L-lactate dehydrogenase B chain are found to be downregulated, while phosphoglucomutase 1 showed upregulation in hippocampus. The identification of these differentially expressed proteins of three distinct regions of schizophrenic brain provides a new insight for a better understanding of pathophysiology of schizophrenia. As the identified proteins are found to be potent metabolic proteins, further characterization will contribute to explore the defective metabolic pathways which may be involved in onset or/and progression of this devastating brain disorder.

Investigation of luminescent properties and antimicrobial activity of synthesized compounds

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Luminescent properties have been studied for methyl endomethylene tetrahydrophthalic acid. Luminescence determination was carried out at room temperature and values of $\lambda = 353$ nm. Luminescence was visually determined. As a result, it was found that all tested substances could be used as blue emission luminophores.

The antimicrobial properties of methyl endomethylene tetrahydrophthalic acid were studied by a serial dilution method, in which a 1% alcohol solution of the test substance was diluted in distilled water to various concentrates (dilution 1:100; 1:200; 1:400; 1:800; 1:1600; 1:3200). Then 0.1 ml of test culture containing 900 thousand microbial bodies in 1 ml was seeded into the test tube with test substances. As a test culture, golden staphylococcus, intestinal stick, synegunous stick, and serration were used. The same dilutions in ethyl alcohol were used as a control but in the absence of test substances. Seeding was done after 10 minutes for an hour.

Studies have shown that the test dibasic acids of the hydroaromtic series generally have antimicrobial activity. It should be noted that they have the most pronounced effect on the sineguous stick and serration, as they were observed in a very strong dilution of the preparation (1:1600). They are less active concerning intestinal stick and golden staphylococcus, as their destructive effect is found in dilution of 1:800 and 1:200, respectively, with a mixture of 3-methyl and 4-methyltetrahydrophthalic acids having less effect than each acid alone.

The antimicrobial activity of these acid derivatives has been studied. As test cultures were used the same gram-positive and gram-negative bacteria. The studies were carried out according to the procedure used in the hydroaromatic series of dicarboxylic acids. 96% ethyl alcohol, rivanol, chloramine, furaciline were taken for control.

Studies have shown that monoesters are more active antimicrobial preparations than the dicarboxylic acids from which they were derived, and with the extension of the alkyl group of the monoester, its destructive effect will increase. Thus, for example, to destroy the golden staphylococcus and intestinal stick, the dilution of 4-methyltetrahydrophthalic acid should not be 1:200, to completely suppress these bacteria, the dilution of monoethyl ester should be 1:400, and in monopropyl and mono n-butyl esters, even 1:1600.

The same intensive effect of the monoesters is on the ratio of sineguous stick and serration.

Thus, studies have shown that among of al the test materials, the highest bactericidal activity is exhibited by monoesters of dicarboxylic hydroaromatic acids, which in this respect are superior to the acids from which they were derived.

Oxidative nitrosative and carbonylated stress in patients with β-thalassemia

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β-thalassemias are hereditary hemoglobin diseases that are transmitted in an autosomal-recessive manner and are associated with a decrease (β+) or complete absence (β°) of synthesis of β globin chains and result from a synthetic defect of - β globin chain, as a result of mutation of β gene. There are about one million carriers of thalassemia disease in Azerbaijan and therefore the relevance of the study of this pathology remains to this day.

It is known that β-thalassemia is characterized by oxidative stress, which is a consequence of iron overload due to frequent blood transfusions, which constitute the only treatment for the disease. Production of free radicals causes nitrosative and carbonylated stress.

The aim of this study was to evaluate the significance of nitrosative and carbonylated stress in patients with β-thalassemia.

For the purpose of this study the levels of nitrosative and carbonylated stress status were measured in blood serum of patients with β-thalassemia.

The blood of 91 patients was examined and patients were divided into 2 groups: I group consisted of 46 patients with heterozygous form of β-thalassemia and II group included 45 patients with homozygous form of β-thalassemia. Control group consisted of 10 healthy patients. Levels of biochemical parameters (hemoglobin, direct and indirect bilirubin, total protein and serum iron) were determined using the "Diasys" kits. The nitrotyrosine concentration was measured by ELISA method with the commercial kit "Hycult Biotech". Nitrogen oxide was determined with "R&D Systems" kit. The levels of status were determined with "Carbonyl Proteine" kit from "Immunodiagnostik" (Biotech) company. Statistical analysis was performed with the help of Mann–Whitney test. Statistical significance was determined at $p<0,05$.

In patients with homozygous form of β-thalassemia an increase in direct and indirect bilirubin was found to be by 2 and 1,5 times in both groups, respectively. The concentration of NO increased in both groups ($p<0,05$). A significant increase in the level of nitrotyrosine was found at 63,2±7,3 μmol/l and 75,1±1,7 μmol/l in I and II groups, respectively, against the control at 0,7±0,03 μmol/l. When studying the concentration of carbonylated proteins, it was revealed that there was an increase by 1,5 times in I group and by 2,7 times in II group.

It can be assumed that changes in the system of nitric oxide synthesis and in the formation of low molecular weight and high molecular weight nitrosothiols in patients with β-thalassemia can affect the production of reactive forms of nitrogen and the level of free glutathione, as well as the functional state of erythrocyte proteins involved in nitrosylation processes. Carbonylation of serum protein was significantly higher in thalassemia subjects on regular blood transfusion. It was be assumed that oxidative stress can cause carbonylation of serum proteins and can be used as indicator of duration of transfusion therapy.

EFFECTS OF DROUGHT ON THE ACTIVITIES OF RBP-CARBOXYLASE AND CARBONIC ANHYDRASE IN EAR ELEMENTS AND FLAG LEAVES OF WHEAT GENOTYPES

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According to UN experts, the world population will reach 9.3 billion by 2050 and along with global climate change, it will have a negative impact on natural resources. Considering the population need for high-quality food, clean air, water, land, and a healthy ecosystem, the UN Food and Agriculture Organization claims that, food production will be 40% higher by 2030 and 70% higher by 2050 compared with the current level. As preventive measures, it is important to study the role of some enzymes (RBP carboxylase –RBPC, carbonic anhydrase-CA) involved in the primary carbon assimilation in increasing plant tolerance against abiotic stress factors. Activities of the enzymes, gene expression and protein content are regulated differently depending on the stages of the plant development and adverse environmental factors. If the assay conditions of the enzymes change their kinetic properties, it indicates the regulation at the post-translation level. In this regard, the creation of wheat varieties for human nutrition can be one of the main goals of modern and future biotechnological research. The activities of RBPC and CA were studied comparatively during the milk ripening and wax ripening phases in flag leaves and ear elements of durum (Garagylchyg and Barakatli) and bread (Azamatli and Giymetli) wheat genotypes grown under field conditions. The RBPC activity was almost the same in control and drought-exposed plants during the initial phase. But during the milk ripening stage, the CA activity increased in flag leaves of the tolerant genotypes (Barakatli and Azamatli) under drought and decreased in all studied genotypes during the wax ripening stage. In ear elements, the activities of both enzymes were higher in the awn than in the scale. But during the wax ripening stage, they changed more in the scale. Activities of both enzymes were higher in flag leaves compared with ear elements and towards the end of the wax ripening stage, these activities became higher in the awn and scale compared with the flag leaf. The higher activity of CA in the awn and scale compared with ripening grains is attributed to the fact that it is more involved in photosynthetic CO2 assimilation in ear elements. Changes in RBPC and CA activities in the studied genotypes depended on the stages of development and the effect of drought stress. Due to the gradually imposed drought stress in the field, it is assumed that the reduction of photosynthetic CO2 assimilation limits the distribution and consumption of assimilates. Based on the results obtained, the effects of drought stress on the protein content of the Calvin-Benson cycle enzymes are different in various photosynthetic organs of the plant. Thus, the protein content of the studied Calvin-Benson cycle enzymes did not change in stressed plants under moderate drought, however, their content decreased sharply in the initial ear. This suggests that the protein content of these enzymes in the ear elements is lower than in the flag leaf and drought more affects the Calvin-Benson cycle in the ear elements. Under slight drought, both enzymes have similar activities in stressed and watered variants of drought-tolerant genotypes. However, due to the decrease in RWC below 70-80%, their activities decreased sharply and this decrease occurs earlier in drought-sensitive genotypes. Thus, activities of RBPC and CA in flag leaves and ear elements of wheat genotypes grown in the field under watered and drought conditions increased at the beginning of the grain filling stage and decreased at the end of this stage. But this decrease was observed earlier in the flag leaf compared with ear elements. This suggests that photosynthesis is more active in ear elements during the late stages of grain ripening, and it has a positive effect on grain yield.

Greening of urbanized areas in Absheron peninsula as a conservation factor of herpetofauna biodiversity

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Abstract

Nowadays urbanization is an irreversible process that intensively takes place all over the world. In connection with the increase of population, new cities and towns are being built, therefore, wild species of fauna and flora undergo anthropogenic influence.

We have studied that how the adaptation of the Caspian bent-toed gecko, the Mediterranean turtle and the aquatic snake in the urbanized territories of the Absheron peninsula takes place. It has been established that landscaping of the peninsula is a reliable shelter for mentioned animals and in order to preserve the biodiversity of these reptiles it is necessary to strengthen this process (landscaping) along the main roads and urbanized areas of Absheron peninsula.

Keywords: urbanization, herpetofauna, biotope, technogenic

Introduction

Urbanization is an irreversible process that is intensively occurs in the world. As the population grows new cities and towns are being built so that both the wild fauna and the flora are severely deformed by anthropogenic effects. This process more intensively occurs especially in cities with a rapidly growing population, such as Tashkent, Moscow, and Baku [1-3]. In recent years, urbanization in Azerbaijan has been more clearly observed especially in Absheron peninsula. The reason for that is the favorable natural and geographical climatic conditions, the richness of the underground and surface resources, as well as being the center of science, culture and industry. In recent years the studying of sustainable ecosystems in urbanized areas has become an actual problem of biology and ecology. This also is one of the important prerequisites that plays an essential role in protection of biodiversity in these biotopes [4, 5]. Reptiles in urbanized areas tend to be synanthropic by gaining some features, take part in spreading of some zoonotic diseases or migrate to relatively favorable biotopes. In terms of evolution the urban environment is novel for the reptiles, and its nega-

Materials and methods

The aim of this scientific research is to study the adaptation and diversity of reptiles that live in Absheron's greenery areas renovated in 2015-2019, especially in parks of newly built and commissioned residential areas around the highways Baku-Rostov, Baku-Ganja and also in the territory of peninsula. For this purpose, three reptile species that were characteristic for the peninsula have been studied as background species. These species are Caspian bent-toed gekco - *Tenuidactylus caspius Eichwald, 1831*, Mediterranen turtle- *Testudo graeca L. 1758*, and water snake - *Natrix tessellata L.1758.* The routing method was used during the research. Observations were conducted in the spring, summer and autumn seasons.

Results and discussion

Population growth in Absheron causes migration of wild fauna from biotopes that have been inhabited for many years, gaining new signs of adaptation and potentially leads to destruction of fauna. Therefore, zoologists and ecologists conduct research and make practical suggestions for the protection of endangered species in the population. In Absheron condition the impact of urbanization on herpetofauna can be divided into two groups: The first is anthropogenic factor, which is directly related to human activities, and the second is a technological factor that can be subdivided into two subgroups: a) Factor that is related to industrial enterprises, including the oil and gas industry of Absheron b) Factor of noise caused by ever-increasing passenger vehicles, trucks, and railway trains. From the background species learned in conditions of urbanization the Caspian bent-toed gecko is well-adapted to mentioned factors. It is most common in roadside greenery areas and in technically contaminated areas. Mediterranean turtles are often found in greenery along the highways, especially along the Baku-Rostov and Baku-Ganja roadsides. It is likely that the Mediterranean turtles have a sufficient source of nutrients, they can be easily protected from their predators and have high reproductive intensity. The adaptation of water snakes in these territories is not so noticeable because ponds and puddles have dried up in these areas and their food supply has been depleted. They migrate to different directions from their living places. The anthropogenic and technogenic factors we have mentioned have a greater impact on water snakes. Water snakes are also rarely encountered in the greenery areas of the peninsula. The negative or positive impact of urbanization on wildlife has a direct correlation with the gaining of adaptation traits. Thus, adaptation traits are related to food source, the natural relief and the flora of the area. The flora of the area is the food source of the studied species (especially Mediterranean turtles), regulates the microclimate of the area, on the other hand, is a suitable shelter for wild fauna for protecting from their predators and hot sun.

Conclusion

Observations have shown that planting greenery areas (regardless of its shape and location) in the peninsula is successful approach for conservation of biodiversity of herpetofauna. Greenery areas, especially along the highways, are very close to natural biocenosis, so the reptile species we have studied are freely and normally reproduced there.

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ABSTRACTS OF
POSTER PRESENTATIONS IN ICAB-2019

Possible association between L55M paraoxonase1 polymorphism and oxidative stress in breast carcinogenesis

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Globally in 2018, rate of cancer incidence and related deaths elevated up to 18.1 million and 9.6 million respectively. Including all types of cancer,breast carcinoma (BC)is the most common cause of incidence (11.6%) and mortality (6.6%) among females worldwide. Discovery of precise biomarker is immediately required to identify novel diagnostic and therapeutic targets. Pakistan has highest rate of BC in Asian region. Improved quality of life and disease free survival is not achieved by current available therapies. It may be due to inaccurate molecular mechanism that defines genetic vulnerability to BC. Among various risk factors oxidative damage pose great stress in development of breast malignancy. Oxidative stress is the accretion of reactive oxygen species (ROS) weakly encountered by antioxidant defense system.Oxidative stress plays apotential role in accelerating irregular cell multiplication. Amount of ROS that are generatedinnormal metabolic conditions are increased during diseasecondition. It affects normal function of signaling pathways, which in turns trigger abnormal cell division. Gene polymorphism of Paraoxonase-1 (PON), an antioxidant enzyme has been focus of study in many life threatening diseases. The purpose of current study is to examine possible association of an antioxidant enzyme with the increasedincidencerate of BC among local population. The correlation of PON and lipid peroxidation is measured in BC patients as compare to normal individuals. L55M gene polymorphism was also observed. These findings deliberate the association of oxidative stress in the progression of BC that maycontribute todevelop better therapeutic strategies.

Keywords: Breast cancer – Gene Polymorphism - Oxidative stress – Paraoxonase1

Phytochemical investigation of bioactive secondary metabolites from *Saussurea lappa* Clarck

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Saussurea lappa C. B. is well known medicinal plant, traditionally it has been used in traditional systems of medicines such as Indian system of medicine Ayurveda[1-4]. Roots of *S. lappa* are commonly used in treatment of gastric issues, arthritis bronchitis, asthma and inflammation related diseases. Phytochemical studies of roots of *S. lappa* led to the isolation of two new compounds, 12-hydroxy-15-methylhexadec-9-enoic acid (**150**) and 11-hydroxy-13-methyltetradec-9-enoic acid (**151**) and a known compound Inulin[1] (**2**). To elucidate the structures of these compounds, UV, IR, EIMS, 1D (^{1}H-NMR and ^{13}C-NMR) were used as well as comparison with those of reported compounds of related structures. Eighteen compounds were identified through GC/GCMS as eight hydrocarbons (**23, 48, 49, 51, 66, 77** and **79**), five terpenes (**6, 9,11,27** and **28**), an alkaloids (**34**), an oxide (**130**), two ketones (**96, 113**) and an alcohol (**19**). Compounds **9, 19, 23, 48, 49, 51 ,66 ,77, 79** and **113** are reported as antibacterial agents in literature whereas **79** as antifungal agent.

Key words: *Saussurea lappa,* root, antimicrobial activity, antifungal activity

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Phytochemical and antimicrobial investigation of anthers and roots of *Bombax ceiba* Linn.

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Bombax ceiba Linn.commonly known as silk cotton tree (Sumbal) belongs to family Bombacaceae[1-3]. Phytochemical studies of anthers of flowers and roots of *B. ceiba* Linn. led to the isolation of five chemical constituents (**1-5**). The chemical examination of anthers of flowers afforded of four chemical constituents, protocatechuic acid (3, 4-dihydroxy benzoic acid, **1**), 3, 4-dimethoxy cinnamic acid (**2**), 4-methoxy cinnamic acid (**3**) and Isovanillic acid (3-Hydroxy-4-methoxy benzoic acid, **4)**. No any phytochemical investigation has been reported from anthers of this plant, therefore, all these compounds are known constituents but new from anthers of flowers. While the chemical investigation of dried roots of *B. ceiba* has led to the isolation of known compound lupeol (**5**). To elucidate the structures of these compounds, UV, IR, EIMS, 1D (^{1}H and ^{13}C) and 2D-NMR (COSY-45°, NOESY, HMBC, HSQC) were used as well as comparison with those of reported compounds of related structures. Fifty two compounds (**5-56**) were recognized through GC/GCMS analysis of antibacterial and antifungal active non-polar fractions of roots and anthers of the plant and also explored the compounds responsible for these activities.

Key words: *Bombax ceiba*, anther, roots, antibacterial activity, antifungal activity

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Isolation, characterization, GC/GCMS profile and antimicrobial activity of leaves of *Daucus carota* Linn.

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Daucus carota Linn. (carrot) is a biennial, white flowering plant and a member of the family Apiaceae (Umbelliferae). It is familiar worldwide due to its root which is used for both food and medicinal purposes. The roots have been used in Ayurveda, Unani, Siddha as well as traditional Chinese medicine for the treatment of ancylostomiasis, dropsy, chronic kidney disease and bladder affliction. It increases the quantity of urine and helps in elimination of uric acid, and exhibits beneficial effect in treating cognitive dysfunctions. It also possesses hepatoprotective, hypoglycemic, antitumor antiulcer and anti-inflammatory activities. Present phytochemical investigation on methanolic extract of leaves of *D. carota*led to the isolation of a new coumarin *C*-glucoside (**1**) along with maltol glucoside acetate (**2**), sucrose (**3**), luteoline 7-O *β*-D-glucoside (**4**), *myo* inositol (**5**), 3, 4-dimethoxy benzaldehyde (**6**), laserine (**7**) and 2-epilaserine (**8**). Compounds **2** and **6** were first time isolated from *D. carota.* The ethanolic extract (DCLE) and their fractions were subjected to evaluate their antimicrobial activity of which some fractions possessed potent potential against tested microbes. The active fractions (DCLEH, DCLEHE, and DCLEEA) were analyzed through GC/GC-MS and explored their active chemical constituents. Over all one hundred and five compounds were identified of which ethyl 9-oxo-nonanoate (**10),** 6,10,14-trimethyl- 2-pentadecanone (**13),** methyl palmitate (**15),** 3,7,11,15-tetramethyl-1-hexadecen-3-ol (**16),** palmitic acid (**18),** 3,7,11,15-tetramethyl-2-hexadecen-1-ol (**25),** γ-sitosterol (**38)** and mono (2-ethylhexyl) ester of 1,2-benzenedicarboxylic acid (**68)** have been reported to have antimicrobial action and may be responsible for the observed activity against tested microbes.

Key words: *Daucus carota,* coumarin *C*-glucoside, GC/GCMS, antimicrobial activity

Biotransformation of Pharmaceuticals for Cost-Effective Wastewater Treatment

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Contamination of water resources with pharmaceutical residues and active compounds has emerged out as a serious concern of this century. Unnecessary persistence of these compounds including antibiotics has been related to the increased risk of resistance selection among pathogenic and non-pathogenic microorganisms which indirectly influence the mortality and morbidity rates associated with the infections caused by them. Till date several methods have been devised to eliminate such pollutants from wastewater but their implication on larger scales is not feasible due to complexities and high costs of the processes. Therefore, this study was designed to develop a simple and cost-effective method for the removal of antibiotics from wastewater with the aid of microorganisms having degradation potential towards them. Isolation, screening and identification of antibiotic resistant bacterial strains was carried out from domestic and pharmaceutical effluents followed by screening and MIC determination experiments against a number of antibiotics. Culture conditions including incubation temperature, initial pH, inoculum size and growth time were optimized and biotransformation efficiency was evaluated by a microbiological assay for the selected bacterial strains, which were then subjected to molecular characterization by 16S rRNA sequence analysis. Of all the isolates, two bacterial strains, namely *Comamonas jiangduensis and Aeromonas hydrophila* showed highest resistivity against two antibiotics Erythromycin and Sulfamethoxazole-trimethoprim. Almost complete biotransformation of both antibiotics was achieved within 92 h of growth under optimum growth conditions indicating microbial use as an alternative for wastewater treatment to eliminate antibiotic pollution.

KEY WORDS:

Antibiotic resistance

Biotransformation

Comamonas jiangduensis

Proteus vulgaris

Aeromonas hydrophila

Green synthesis of puerarin coated gold nanoparticles (PUE-AuNPs): A colorimetric probe for ciprofloxacin

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Abstract

Ciprofloxacin (CP) is one of the most widely used second generation fluoroquinolone, broad spectrum antibiotic, due to its wide range of antibacterial activity. It has been gradually found in water sources which has been shown to be very harmful for environment. In aquatic ecosystem, antibiotic contamination has harmful effects on un-target organisms, like preventing algae growth, facilitating bacterial resistance, and damaging chloroplast replication, interrupting the micro-organism N cycle, and translation and transcription. Here PUE-AuNPs, a new colorimetric sensor was synthesized using secondary metabolite puerarin, an isoflavonoid compound through a green synthetic route. This sensor efficiently detected the CP in tap water and cow milk samples. The PUE-AuNPs were characterized by UV-Vis, FTIR, AFM, and DLS techniques. The PUE-AuNPs were found to be spheroid with an average size of approximately 19-20 nm. FTIR spectrum evidences the specific functional groups such as –OH, >C=O, -CO and >C=C< were responsible for the reduction of Gold (III) chloride trihydrate and acted as capping agents to form AuNPs. The PUE-AuNPs sensor was selectively detected CP in the presence of other interfering drugs. The sensor was proved to be selective and sensitive for the detection of CP with in the concentration of 1 to 1000 μM and the limit of detection was 51 μM. Moreover, AuNPs, showed good antifungal effect specifically *Penciillium spp.* (ZI = 36.0 ± 0.8 mm) as compared to standard drug Nystatin (ZI = 21.0 ± 0.8 mm). This colorimetric sensor is simple, cost effective, and selective towards CP detection in various environmental samples.

Keywords

Gold nanoparticles, Puerarin, Ciprofloxacin; Colorimetric sensor; Antifungal activity

Comparison of methods for pretreatment to sugarcane bagasse for thermostable xylanase production using multivariate approach

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Several million tons of sugarcane bagasse (SB) are generated by the sugar industries in Pakistan, which remain under-utilized. The lignin content in SB hinders its utilization by microrganisms, therefore, pretreatment methods are employed to make fermentable components available to the microbes. In the present work, SB was treated with dilute acid, alkali, hydrogen peroxide or ionic liquid and was utilized as a substrate to produce xylanase from a thermophilic strain of *Bacillus aestuarii* UE25. Three Plackett-Burman Designs (PBDs) were executed separately to screen the significant factors affecting submerged fermentation (SmF) of different pretreated SB for the production of xylanase by the bacterial strain. The analysis depicted that SmF of pretreated SB using NaOH in combination with ionic liquid significantly enhanced the enzymatic hydrolysis. Five factors showed up as significant variables (temperature, agitation, pH, inoculum size and incubation time). Further optimization of these significant factors was carried out by adopting Box-Behnken Design (BBD) in Response Surface Method (RSM) approach. The model predicted 20 IU mL^{-1} of xylanase under optimum parameters of 59°C; 7.03% inoculum size; pH 5; agitation 141 rpm and incubation period 24 h in fermentation medium. Practically, a titer of 17.77 IU mL^{-1} was obtained showed the validity of the model. Time course studies of the enzyme production revealed association of xylanase production with the growth and optimum enzyme production was accomplished after 48 h of cultivation. Extractions of xylan and cellulose from SB under alkaline conditions were performed, and this xylan and cellulose were used as substrate for production of xylanase and cellulase. Comparing with commercial substrates, maximum xylanase was produced by utilizing extracted xylan. This study indicated the significance of SB in cost effective production of xylanase by *Bacillus aestuarii* UE25 that may be used potentially for increasing productivity as well as various industrial applications. The results have been encouraging enough to merit further investigation of biotechnological potentials of the strain.

THE STUDY OF THE WATER POLLUTION IN THE WEST OF ALGERIA

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One of the objectives of a modern society is the improvement of the living conditions of the Man. If one goes up the course of the centuries, the history teaches us that the water pollution accompanied all civilizations and that it was always a concern for the legislator until the beginning of the century. Nature, thanks to its intrinsic capacities of regeneration managed to overcome the misdeeds of the man. The pollution developed because of the concentration of the population and the development of her industrial activities. Another time, the pollution water was primarily of organic nature biodegradable never not exceeding the purifying capacities car of the rivers, but the industrial evolution contributed to the marketing. Molecules polluting, that micro the organizations cannot always metabolize and of which some constitute a danger to the Man and his environment. An awakening of an alarming situation as regards quality of water dates from the Seventies. All the types of water were concerned, the subsoil waters, until there with the shelter, are also reached and from now on, one does not cease speaking about nitrates, micro mineral and organic pollutants. Vis-a-vis with this very critical situation at the same time for the health of the man and the environment, the resources water; a vast program of prevention and fight launched out to Algeria in the Seventies/80 and which aimed, the construction of several stations of purification in the most important cities, generally of a size higher than 20. 10' living Following an assessment of this effort of investment established in 1989, it appears clearly that ohjectif stations of purification was unfortunately not reached but on the contrary the situations developed well , then it appears very important to us to draw up through this modest work, a point of topicality on the state of the quality of water in our area of West of Algeria, with an analysis of the causes of the pollution and the search for the solutions to decrease by its impact.

KEY WORDS : Water Resources , Pollution Sources , Protection , Purifying .

Hereditary Glomerulonephritis, Collagen IV-Related Nephropathies &TBMN: X-Linked Alport Syndrome: A Clinical and Genetic Dissection

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Alport Syndrome is a rare genetic disorder characterized by Hematuria, Proteinuria and hearing with a classification number *ICD-10:***Q87.8.** X-Linked Alport Syndrome is a hereditary glomerulonephritis disorder resulting from mutation (s) point or intragenic deletions of the *COL4A5 gene* encoding the α5 chain of type IV collagen. X-Linked Alport Syndrome (XLAS), autosomal recessive Alport Syndrome (ARAS), autosomal dominant Alport Syndrome (ADAS) and TBMN (Thin Basement membrane) comprise a spectrum of phenotypes of collagen IV-related nephropathies subtypes. Alport Syndrome is characterized by renal, ocular and cochlear involvement. Renal diseases progress from microscopic hematuria to end stage renal disease (ESRD) in all males and females with ARAS.Sensoneural hearing loss (SNRL) by late childhood and TBMN. As a rare disease, the prevalence of Alport syndrome is not well-known, although it is estimated to be 1 in every 50,000 live births worldwide. In United States, it is believed to affect 1 in every 5,000 people, while across Europe the estimated range may vary from 1 in 100,000 people to 1 in every 11,000**.**The diagnosis of the disease is based on family history, clinical signs and the results of renal biopsy, showing abnormalities of the glomerular basal membrane by electronic microscopy examination. The study of the binding of antibodies directed against the alpha 3, alpha 4 and alpha 5 chains of collagen IV in the kidney and skin also allows the diagnosis to be made.

Key Words: Hematuria, Proteinuria, autosomal dominant, autosomal recessive

Biotransformation of Androstanes by Using *Curvularia lunata* and *Curvularia clavata*

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The structural changes in a substance by using microorganism (bacteria, fungi and algae) is called as microbial transformation. It is an effective tool to synthesize many steroidal drugs with potential biological activities. Microbial transformation of steroids is a traditional field and has been extensively investigated in industry. The reactions involved in microbial transformation are oxidation, reduction, hydrolysis, hydroxylation, epoxidation and formation of new carbon bonds.

Androsta-4-ene-3,17-dione (**1**), 17aβ-hydroxy-17aα-methyl-D-home androstane-3,17-dione (**2**),

16α,17α-epoxy-5α-androstan-3-one (**3**) and 16β,17β-epoxy-5α-androstan-3-one (**4**) are androstane derivatives. They are responsible for sex determination, fetal development, growth and sexual maturation and transformation. Several transformed products **5**-**17** were obtained by using *Curvularia lunata* and *Curvularia clavata* from substrates **1**-**4**.

Key words: Microbial transformation, Androstanes, *Curvularia lunata*, *Curvularia clavata*

IN-VITRO STUDIES OF THE ANTIBACTERIAL ACTIVITY OF *OCIMUM SANCTUM* AND *AZADIRACHTA INDICA* ON MRSA, MSSA AND VRSA

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Medicinal plants have been used since several 1000 years. Plants' parts including seeds, root, stem, leaves, and fruits mainly comprising of bioactive components that have been approved for primary health care. *Ocimum sanctum* (Tulsi) of the family Lamiaceae and *Azadirachta indica* (Neem) are considered as herbal therapy having antibacterial and antiviral properties when used in different concentrations.

This study indicates the antimicrobial properties of Tulsi and Neem leaves extracts against the Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin sensitive *Staphylococcus aureus* (MSSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) and analyze the zone of inhibition and spectrophotometric studies of extracts at variable concentrations. The test culture were grown on Brain Heart Infusion agar and broth, one milliliter of fresh culture was mixed with variable concentrations of ethanolic and methanolic extracts in Mueller Hinton agar wells. The zone of inhibition on MHA plates were observed and spectrophotometry 600 nm was performed after 24 hours incubation.

The observation revealed greater antibacterial effect of ethanolic extracts on all test strains at 10, 8, 12% of Neem whereas 14, 10, 12 % concentration of Tulsi on MSSA, MRSA and VRSA clinical isolates respectively against the standard antibiotic discs Methicillin and Vancomycin. These observations showed that the Neem and Tulsi have significant effect on test strains and can be used by pharmaceutical industries and at home therapy in the form of juice or paste against Staphylococcal infections.

Key words.

Antibacterial activity, Staphylococci, Neem, Tulsi, disk diffusion method, Spectroscopy

ISOLATION AND IDENTIFICATION OF AQUATIC MICROBES FROM VARIOUS SOURCES OF WATER OF HYDERABAD CITY

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Water is an important component of life, though it is life for all living organisms including the microorganisms such as bacteria, viruses, protozoa and fungi. They live normally or they live as pathogenic flora, cause water pollution, spread health risk for humans or animals with the inclusion of all the illnesses and severe water-borne diseases. Hyderabad, the second largest and populous city of Sindh Province, which is facing critical problems of drinking water that may be contaminated through various sources of wastewater of Hyderabad city carrying aquatic pathogens, in-organic and organic matters and metallic compounds. The people of various areas are using water from various canals that are not properly treated and extend the pathogens that may the cause more or severe illness all ages and both sexes.

This presentation elaborates the numbers and type of aquatic pathogens per milliliter of samples. The study 42 water samples revealed higher and lower numbers of bacteria including 94,00,000 and 34,00.000 (Hirabad market); 98,00,000 and 29,00,000 (Railway station and hotels) 91,00,000 and 21,00,000 (Park canteens); 64,00,000 and 44,00,000 (Qasimabad hotels); 82,00,000 and 49,00,000 (Ranibagh Canteen); 94,00,000 and 24,00,000 (Bus stop hotels); 79,00,000 and 24,00,000 (University Canteens). The results revealed *E.coli Klebsiella sp. Pseudomonas sp. Enterobacter sp. Staphylococcus sp. Streptococcus sp.* as aquatic microbial flora in test samples.

Key words. Potable water, Bacterial contamination, G+ve and G-ve isolates

SERODIAGNOSIS OF HBV, HCV AND HIV IN BLOOD DONORS AND PATIENTS OF TALUKA MORO, DISTRICT NOUSHAHRO FEROZ

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Naushahro Feroze is a district of Sindh Province of Pakistan since 1989. The Moro Taluka of this district is comprising of 23 Union councils, population is 327196 and located about 12 km of the Indus River. The population is of mainly poor and average status, which are facing acute health problems. The common diseases prevailing hepatitis, HIV and other infections including syphilis.

Hepatitis B and C virus (HBV) infection is an infection of liver that extends to liver cancer, which are the main causes of mortality worldwide that lead to the Hepatocellular carcinoma and lymphoma in humans. HIV is originated from 1981 that causes acquired immunodeficiency syndrome (AIDS). It has emerged as the greatest threat to human existence that is more prominently extended to millions of population in Africa, Asia and other developed countries since decades. The dissemination of spread of these infections are infected blood transfusion, semen or other body fluids from unprotected sex with multiple sex partners, sharing infected needles, infected mother, saliva, carriers in blood bank and travelling to the areas of endemic and homosexual and heterosexual intercourse.

This work shows the frequency of the occurrence of Hepatitis B, C and HIV infections in Taluka Moro. The two year data collected showed 9.9, 10.9 and 1.4% mortality rate. The laboratory findings revealed HIV (2.3%), HBV (25.5%) and HCV (26.8%) in females and (3.2%), (19.3%) and 18.7% in males at different villages of Taluka Nousharo Feroz.

The observations of collective percentage revealed 31.4% and 27.1% viral infections in male and females respectively. This infection rate is an alarming condition that may increase steadily to the greater morbidity and mortality rate. It is suggested that always wash hands thoroughly before eating food and using blood and body fluids collection tools, get children vaccinated for Hepatitis B and immediately treat hepatitis C patients.

POPULATION BASED STUDY OF FOOD BORNE ANTIBIOTIC RESISTANT BACTERIAL ISOLATES FROM CONSUMERS' FOOD SAMPLES IN HYDERABAD

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Foodborne diseases are more or less severe, which occurs due to the contamination of polluted environment, unhygienic handling and cooking. Microorganisms are the source of food infections and intoxication that cause various stomach complications. Now a days, eating commercially prepared foods is increasing among all classes of communities. The essence of this study is to analyze age, gender wise eating habits, hygienic status, food borne pathogens and their antibiotic resistance.

The findings revealed 4-22 (41%) 23-35 (26%), 36-50 (20%) and 51-60 (13%) are habitual of eating commercial foods. The health issues analysis showed that 17%, 4%; 6%, 4%; 8%, 18%; 8%, 5.5% of male and females of 4-22; 23-35; 36-50; 51-60 years had suffered from various stomach problems. The studies of hygienic measures revealed (27%) wash food items before cooking (15%), wash utensils with detergent and hot water (11%), use oven dried crockery after washing to serve customers (11%). Both G+ve and G-ve bacteria were isolated from 200 food different samples that include *S. aureus* 30, 35, 20%; *Sal. enterica* 35%; *C. jejuni* 20%; *E.coli* 35, 30, 25%; *Str. lactis* 45%; *Ps. aeruginosa* 15%, 5%; *B. cereus* 20%; *L. monocytogenes* 20%.

The antibigram revealed the greater resistance 38%, 22%, 28.5%, 35%, 25%, 37.5%, 36% and 25% of *Staphylococcus aureus, Streptococcus lactis, Listeria monocytogenes, Bacillus cereus, Campylobacter jeuni, Salmonella enterica, E. coli, Pseudomonas aeruginosa* respectively whereas *Shigella dysenteriae* and *Proteus mirabilis* showed no resistance on test antibiotics.

Key words. Demographic studies, commercial foods, food borne pathogens, antibiotic resistance.

In silico Analyses of Hub Genes Associated with Pancreatic Cancer

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Understanding Hub genes (which having a high degree of intra-module connectivity) in pancreatic cancer samples could lead to efficient approaches to diagnose and treat cancer. In this study, Hub genes were identified using bioinformatics tools from microArray dataset of pancreatic cancer.
To explore potential genes (Targets) in pancreatic cancer, two microRNA expression profiles (GSE89120 and GSE85991) were extracted from the Gene Expression Omnibus (GEO) database. The GEO2R online tool was applied to screen out Differentially Expressed Genes (DEGs) from healthy individuals and pancreatic cancer samples. DAVID analysis was applied to perform Gene Ontology and KEGG pathway enrichment analysis. The protein-protein interaction (PPI) network of identified DEGs was constructed using STRING online software. The hub genes were identified by the CytoHubba plugin of Cytoscape software. Then, the prognostic value of these identified genes was verified by pancreatic cancer database derived from Kaplan-Meier plotter platform.
A total of 3 overlapped up-regulated genes and 9 down-regulated genes were identified. The majority of DEGs were significantly enriched in ribosomal activity, trans-membrane transporter, Signal transduction, cell cycle and growth apoptosis. Moreover, KEGG pathways were significantly enriched, in choline metabolism in cancer, Protein processing in endoplasmic reticulum, phosphatidylinositol signaling. By combining the results of PPI network and CytoHubba, a total of nine hub genes including DAP3, MRPS18B, MRPS5, MRPL13, MRPL21, MRPL4, MRPS15, MRPS9 and MRPS2 were selected. The Kaplan-Meier plotter database confirmed that over expression levels of these genes is associated with reduction in overall survival.
Our study suggested that the identified hub genes may be potential biomarkers and therapeutic targets against pancreatic cancer.

Keywords: Hub genes, GEO, In-silico, Pancreatic cancer

Analyzing single nucleotide polymorphism (Gly >Ser) of GRIA1 gene in schizophrenia with respect to Pakistani population.

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Schizophrenia is a mental disorder characterized by abnormal behavior, strange speech, and a decreased ability to understand reality sometimes including delusions hallucinations, poor expressions, lack of motivation, social withdrawal and cognitive difficulties. About 1.5% of the population of Pakistan suffers from schizophrenia. The etiology of schizophrenia is still unknown, but it could be stated that genetics and environmental factors play a significant role. Neurobiology of schizophrenia also involves Impairment of glutamatergic neurotransmission. Therefore, Glutamate Ionotropic Receptor AMPA Type Subunit 1 (GRIA 1) could be considered a substantial contributor in causing schizophrenia. It maps at 5q33, which is a susceptible locus for SHZ as per three independent genome wide scans. The aim of this study was therefore to investigate whether single nucleotide polymorphism (rs1127386, G/A) of GRIA1 is a risk factor for schizophrenia in population of Pakistan. Schizophrenia cases (49) were recruited as per DSMV criteria, and 37 controls were recruited. Genomic DNA was extracted from blood and polymorphism was detected using ARMS (amplification refractory mutation system) PCR. the results were observed using Agarose Gel Electrophoresis. In control group 5.4% individuals were detected as wildtype homozygous (GG) whereas rest 94.6% individuals were heterozygous (AG). In comparison 4 % cases were detected as wildtype homozygous while we found 96% cases as heterozygous. No obvious difference was found in genotype distribution of cases and control. Multi-center study with large sample size is encouraged in future to detect reliable association of this genetic polymorphism of GRIA1 in the schizophrenia patients of Pakistan.

SCREENING OF SINGLE NUCLEOTIDE POLYMORPHISM IN DNA-MISMATCH REPAIR PATHWAY GENE MLH1

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ABSTRACT:

Mut L Homolog1 MLH1 is a key mismatch repair gene that is mutated in a number of cancers i.e., colorectal, colon, lung, breast and ovarian cancer. DNA mismatch repair system genes are closely related to the expansion of different types of cancer related diseases in human by causing microsatellite instability, epigenetic silencing of DNA-MMR Protein and DNA methylation. This study was designed to investigate the Cancer biomarkers i.e. missense variants in MLH1 gene and examined the influence of variations on the structure and stability of protein of MLH1 gene using bioinformatics approaches.

A total of 536 SNPs, 95 were non synonymous (missense) whereas 418 synonymous and 23 non sense non synonymous mutations were found in coding region of MLH1 retrieved by using NCBI Database. Initially SIFT was used which predicted 12 potential missense nsSNPs from 95 nsSNPs in MLH1.These 12 nsSNPs were further analyzed by SNAP2, nsSNPAnalyzer, PolyPhen-2, SNPs&GO, FATHMM and PROVEAN servers.06 potential variations (K618E, L622H, R659P, P648L, P648S, P654L) were expected to have adverse effects on protein .Three dimensional structure of template (3rbn) was downloaded from RCSB Databank PDB. Mutant models of MLH1 were formed using MODELLER 9.16. Overall variants models strength was predicted using SNP effect which shows reduction in structure stability. Furthermore, mutants model were evaluated for structural deviation compare to template by TM Score and ConSurf was used to identify conserved and exposed amino acids in MLH1 protein sequence. All 06 mutant models were showing increase in energy compare to template but R659P showed high increase in energy(-10961.054 KJ/ mol) when compare to the native (-25467.693 KJ/ mol) energy which may effect on overall structure stability. In conclusion, we can evaluate that predicted 6 mutations can influence on MLH1 protein structure, performance and its mechanism. Moreover, this research can give a new strategy to categorize nsSNPs for several important regulatory genes and their association with disease status and further development in potential drug discoveries.

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Characterization of salivary protein profile and oxidative stress status among young healthy smokers and non-smokers

Basma Aziz, Urooj Ishrat, Tehseen Fatima and Sadaf Khan.

Human saliva is a heterogeneous fluid containing several organic and inorganic molecules, including an array of proteins and peptides. The composition of salivary proteins can be affected by several factors including cigarette smoking. Smoking is the most important risk factor for several tobacco related diseases such as oral and lung cancer. Saliva is the first body fluid which gets exposed to cigarette smoke. Cigarette smoke contains several toxins and oxidants and can alter the normal homeostasis of oral cavity which may lead to different oral diseases. The aim of the current study is to characterize the salivary protein profile and oxidative stress status in young healthy smokers and non-smokers. To conduct the study saliva samples are collected from forty healthy young male subjects (20 smokers and 20 non-smokers), aged 18-35 years. Total protein concentration is measured by braddford assay and further oxidative stress status will be measured by reactive oxygen species (ROS) assays. Salivary protein profile will be evaluated by SDS-PAGE. Our study will help in understanding the role of salivary proteins in oral health and pathogenesis and may also help in identify the biomarker for tobacco related oral cavity diseases.

Keywords:

Saliva, Smokers, Oxidative stress, Protein profile, Cigarette smoke

Oral dysbacteriosis in type 2 diabetic mellitus

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We characterized the relationship of oral microbiome with diabetes in Pakistan. Saliva samples were collected from diabetic patients (n = 49) and healthy individuals (n = 55). 16S metagenomics saliva was carried out by NGS technology. We observed that the phylum *Firmicutes* (p-value = 0.024 at 95% confidence interval) was significantly more abundant among diabetic patients than among the controls. We found that abundance of phylum *Actinobacteria* did not significantly vary among both groups in contrast to a similar report from USA (Long et al., 2017). On genus level, acidogenic bacteria *Prevotella* (p-value = 0.024), *Veillonella* (p-value = 0.013) and *Laptotrichia* (p-value = 1.5 x 10^{-3}) were found to be in higher abundance in diabetic patients. Stratified analysis by gender revealed healthy and diabetic females to be more divergent. Abundance of *Prevotella* (p-value = 4.4 x 10-3) and *Laptotrichia* (p-value = 0.015) was significantly associated with male patients. Comparison of oral bacteriome between two groups revealed dominance of acidogenic bacteria in diabetics. These bacteria are found in dental biofilm and produce organic acid(s) from fermentable carbohydrates. Significant abundance of acidogenic bacteria suggested involvement of these eubacteria in periodontal diseases in diabetic patients.

Clinical control of Almond *(prunus amygdalus*) shell extract on different microbial infections

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The Almond *(Prunus dulcis*) shells was confirmed for the preparation of antimycotic extract against microbial mix infections . Clinically studies of this cheap material can be proceed as a phytomedicines. Shell extract prepared by soxhlet method and further characterized by UV-Visible spectrophotometer. The antioxidant activity of shell extract was also evaluated by using DPPH and trace elements by Atomic absorption spectrophotometer. The total antioxidant activity varied from 93.33 to 94.43% and total phenolic content was found 5.465 mg/g in almond shell extract. Finally, the results provide great therapeutic effect against the microbial infections and the al result can be achieved in 12 days of therapy. In this study, scientific evidence shows its safe use as a harmonizing and substitute treatment for the different skin infections.

Keywords: Almond shell; Tinea infection, phenolic constituents; DPPH antioxidant process; Trace elements.

Pan-genome Analysis of Hypervirulent *Klebsiella pneumoniae* to Evaluate Genomic Diversity and Pathogenic Potential Among Strains

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The aim of this study is to perform the pan-genome analysis of *Klebsiella pneumoniae* to reveal the genetic variation among its different strains. It is the causative agent of numerous types of infections in humans, includes urinary tract infections (UTIs), respiratory tract infections, and bloodstream infections. Several worldwide reports show that *Klebsiella pneumoniae* is a leading infection-causing bacterium and causes numerous outbreaks.

In the present study, we have performed the pan-genome analysis of *Klebsiella pneumoniae* for the determination of virulence proteins and genomic variation among its strains using a tool pan-genome analysis pipeline (PGAP-1.2.1). To initiate the analysis, we obtained the whole genome sequences of fifty-one *Klebsiella pneumoniae* strains from NCBI using Refseq database. Based on the literature survey thirty-nine proteins sequences involved in the pathogenesis of the organism were downloaded from UniprotKB for BLAST analysis. Following the preparation of input data files, pan-genome analysis pipeline was executed.

The output file generated by pan-genome analysis pipeline (PGAP) which contain orthologous clusters data were segregated into core, shared and unique genome. It was observed that a wide variety of selected pathogenic proteins were present in core and shared genome which includes siderophores specifically (salmochelin), adhesion proteins, extracellular toxins and complement resistant proteins. Moreover, presence of carbapenem resistance and hypermucoviscosity proteins in unique genome indicated that *Klebsiella pneumoniae* is continuously evolving through horizontal gene transfer (HGT).

Pan-genome profile curve and new gene curve were constructed using a tool PanGP thus it was observed that *Klebsiella pneumoniae* has an open genome due to the subsequent increase in the pan-genome with the addition of new genomes.

Thus, it is concluded on the basis of presence of virulence proteins that hypervirulent *Klebsiella pneumoniae* is an emerging superbug and there is no diagnostic technique available in microbiological laboratories to identify and differentiate between classical cKP and hypervirulent hvKp strains of *Klebsiella pneumoniae* therefore further investigation and development of diagnostic techniques for hypervirulent *Klebsiella pneumoniae* should be done. Moreover, antibacterial drugs could be designed by utilizing the siderophore transport system present in outer membrane of *Klebsiella pneumoniae.*

Green synthesis of puerarin coated gold nanoparticles (PUE-AuNPs): A colorimetric probe for ciprofloxacin

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Abstract

Ciprofloxacin (CP) is one of the most widely used second generation fluoroquinolone, broad spectrum antibiotic, due to its wide range of antibacterial activity. It has been gradually found in water sources which has been shown to be very harmful for environment. In aquatic ecosystem, antibiotic contamination has harmful effects on un-target organisms, like preventing algae growth, facilitating bacterial resistance, damaging chloroplast replication, interrupting the micro-organism N cycle, and translation and transcription. Here PUE-AuNPs, a new colorimetric sensor was synthesized using secondary metabolite puerarin; an isoflavonoid compound through a green synthetic route. This sensor efficiently detected the CP in tap water and cow milk samples. The PUE-AuNPs were characterized by UV-Vis, FTIR, AFM, and DLS techniques. The PUE-AuNPs were found to be spheroid with an average size of approximately 19-20 nm. FTIR spectrum evidences the specific functional groups such as –OH, -C=O, -CO and –C=C were responsible for the reduction of Gold (III) chloride trihydrate and acted as capping agents to form AuNPs. The PUE-AuNPs sensor was selectively detected CP in the presence of other interfering drugs. The sensor was proved to be selective and sensitive for the detection of CP with in the concentration of 1 to 1000 μM and the limit of detection was 51 μM. Moreover, PUE showed good antifungal effect specifically *Penciillium spp.* (ZI = 36.0 ± 0.8 mm) as compared to standard drug Nystatin (ZI = 21.0 ± 0.8 mm) but its AuNPs did not show activity against any fungal species. This colorimetric sensor is simple, cost effective, and selective towards CP detection in various environmental samples.

Keywords

Gold nanoparticles, Puerarin, Ciprofloxacin; Colorimetric sensor; Antifungal activity.

GENETIC POLYMORPHISM OF THE *UHRF1BP1* GENE ARE RISK FACTORS FOR INFLAMMATORY REACTIONS IN LEPROSY

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Objective:

The objective is to identify genetic variation(s) in *UHRF1BP1* gene and to find out the association of single nucleotide polymorphism in leprosy.

Material and Methods:

Blood samples of 150 leprosy patients from Marie Adelaide Leprosy Centre will be collected after taking written informed consent and compared with age and sex matched controls. Genomic DNA will be extracted through phenol-chloroform method. Genetic association will be identified through Tetra- Amplified Refractory Mutation System Polymerase Chain Reaction (t-ARMS PCR) by using allele specific primers. Genotypic analysis will be performed in order to find out the genetic association with leprosy and data will be analyzed through statistical tools.

Results:

In this study, it is expected that in *UHRF1BP1* gene there might be a missense variation on exon 21 at position 78. This missense variation may replace Thymine into Cytosine at gene level and Methionine into Threonine at protein level.

Conclusions:

The current study might be helpful for the better understanding of molecular mechanism involved in leprosy and may act as a prognostic marker for the early diagnosis of the disease.

Metagenomic characterization of bacterial communities in drinking water supply system of a mega city

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Supplying safe water to consumers is vital for protection of public health. With population of >15 million, Karachi is the main economical hub of Pakistan. Lake Keenjhar serves as the main source of fresh water while Hub dam is secondary water reservoir for Karachi. In this study, bacterial community of the drinking water supply system (DWSS) of Karachi was studied from source to tap using metagenomics approach. For this purpose, we collected 41 water samples from different areas of the city (n=38) and water reservoirs (n=3). 16S rDNA metagenomic sequencing of water samples revealed that 88% sequences were associated with *Proteobacteria* (52%), *Planctomycetes* (15%), *Becteroidetes* (12%) and *Verrucomicrobia* (6%). On the class level, *α-proteobacteria* (6-56%) were found to be most abundant followed by β- (8-41%) and *γ–proteobacteria* (6-52%). On the genus level, substantial diversity was observed among the samples. Bacterial communities in water from Hub dam was found to be distantly related while among the residential towns, Lyari was highly distant from the others. 24 bacterial genera were found to be exclusively present in residential areas samples in comparison to the source waters which is suggestive of their resistance against treatment procedures and/or contamination. Metagenomic analysis revealed abundance of *Pseudomonas*, *Legionella*, *Neisseria*, *Acinetobacter, Bosea* and *Microcystis* genera in residential areas water samples. The present metagenomic analysis of DWSS of Karachi has allowed the evaluation of bacterial communities in source water and the water being supplied to the city. Moreover, measurement of heavy metals in water samples from Karachi revealed arsenic concentration according WHO standards which is in contrast of recent study which reported extensive arsenic contamination in aquifers in the Indus valley plain. To the best of our knowledge, this is the first metagenomic study of DWSS of Karachi.

Metagenomic evaluation revealed salivary dysbacteriosis in betel-nut preparation chewers

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Betel nut addiction is recognized as the causative agent of oral microbiome dysbiosis and other systematic disorders. Despite this, the underlying variations in the oral microbiome due to usage of betel nut preparations such as gutka are poorly understood. In this study, 16S rDNA metagenomic analysis of salivary microbial communities in Gutka chewers (n = 16) and non-chewers (n = 55) was carried out. It was observed that Gutka chewers demonstrated lower alpha diversity and number of bacterial genera than the non-chewers. Taxonomic assignment on phylum level revealed *Firmicutes* (p-value = 0.042 at 95% confidence interval) to be significantly more abundant in Gutka chewers in comparison with non-chewers. Beta diversity on genus level unveiled both groups to be divergent from each other. On the genus level, *Veillonella* (p-value = 0.015), Streptococcus (p-value = 0.026), *Laptotrichia* (p-value = 0.022) and *Serratia* (p-value = 0.022) species appeared to be significantly more abundant in Gutka chewers in comparison to non-chewers. *Veillonella* species are aciduric in nature, which thrive and adhere to oral cavity by utilizing organic acids produced by acidogenic bacterial genera (i.e. *Streptococcus* and *Laptotrichia*). This observation is an indicative of dental caries development in Gutka chewers. The present study contributes additional information regarding oral microbiome variations with response to gutka consumption.

Pairwise Dissolution Profile Comparison of Newly Designed Propranolol Floating Tablets Using *Prunus Domestica* Gum

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Propranolol HCl is a beta adrenergic blocker commonly prescribed for the treatment of hypertension. The purpose of present study was to explore the role of *Prunus domestica* gum with HPMC on the dissolution kinetics of Propranolol floating tablets by difference (f1) and similarity factors (f2). A full factorial design (2^2) was applied; tablets were formulated by Direct Compression (DC) method and evaluated by official and unofficial tests. Dissolution was carried out in USP Apparatus II (using 0.1N HCl), samples were drawn at different time intervals and analyzed by spectrometry. All the formulations complied official guidelines. The formulation F2 showed highest dissolution rate and was considered as a reference in present study. On the basis of f1 and f2 factors acceptance criteria, the formulations F1 and F4 were accepted while F3 was rejected. Pairwise comparison is easy to compute but it is recommended to further analyze the data by other statistical methods before deciding similarity of dissolution profiles.

Keywords: *Prunus domestica*, Floating tablets, Pairwise procedure, Dissolution

To Determine The Pattern Of Fosfomycin Sensitivity In Clinical Isolates Of Staphylococcus Aureus

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Fosfomycin is an old antibiotic with a unique chemical structure and with broad-spectrum activity against numerous bacterial pathogens, both Gram-positive and Gram-negative, including resistant and multi-resistant strains. This antibiotic found its way into clinical practice in the early 1970s.Its use, however, has been limited for several years for treating mainly lower uncomplicated urinary tract infections. Fosfomycin also has an intravenous formulation (Fosfomycin disodium) which achieves clinically relevant concentrations in the serum and the cerebrospinal fluid, in kidney, bladder wall, prostate, lungs, bone and heart valves tissues as well as in inflamed tissues and abscess fluid.To determine the pattern of Fosfomycin sensitivity in clinical isolates of staphylococcus aureus.The study was conducted over 4 month's period (August 2015 to November 2015) in department of Microbiology Sindh Institute of Urology and Transplantation. A total of 150 consecutive clinical isolates 0f Staphylococcus aureus were identified and antimicrobial susceptibility was done as per standard protocol.Out of 150 consecutive clinical isolates of Staphylococcus aureus, 46.66%(n=70) were MRSA and 53%(n=80) were MSSA. 114/150(76%) Staphlococcusaureus were susceptible to Fosfomycin by disk diffusion method. 64%(45/70) of MRSA and 86% (69/80) of MSSA were sensitive to Fosfomycin. 76%(95/124) of Staphlococcusaureus from blood were sensitive to Fosfomycin, Urine 83%(5/6), Pus 73%(11/15) and sputum 60%(3/5).The study suggests that Fosfomycin has reasonably good in-vitro activity against common urinary isolates and it holds true beyond urinary pathogens. Fosfomycin has emerged as a promising option, in which previous antibiotics have failed to cure the infection.

Extraction, purification and characterization of milk coagulant enzyme from different parts of *Moringa oleifera*

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Proteolytic enzymes also known as peptidases, proteases and proteinases have the capability of hydrolyzing the proteins peptide bonds. Proteases obtained from plant sources are widely used in different industries including food industry. It has been reported that Moringa oleifera is a great source of milk coagulating enzyme. This study aims to characterize the caseinolytic and milk-coagulating activities of different parts of Moringa oleifera. For the study, milk coagulant enzyme is extracted from leaves & flowers of Moringa oleifera by ammonium sulfate precipitation method. The caseinolytic activity of the extracted enzyme will be determined using casein as substrate. The molecular weight of the extracted enzyme will be determined by SDS-PAGE and protease activity will be checked by zymography. Effect of different temperature and pH on enzyme stability and activity will also be determined. Exploring the ability of different parts of Moringa oleifera to coagulate the milk can help in determining the utility of this plant in cheese making industry.

COMPARATIVE EFFECTS OF AVOCADO (SEED & PULP) ON D-GALACTOSE INDUCED BRAIN AGING IN ALBINO RATS

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Aging is a well-known physiological process of getting older. It has a negative impact on functional abilities of an individual leading to detrimental deficits like cognitive dysfunction. Many factors contribute in brain aging particularly mitochondrial dysfunction characterized by a decrease in the activity of respiratory chain enzymes and ATP production leading to increased free radical generation, mitochondrial deoxyribonucleic acid (DNA) mutations, and impaired mitochondrial structures. D-galactose induced rodent brain aging model, used by previous researchers, is developed by progressive deterioration of brain cells via mitochondrial dysfunction increased oxidative stress, inflammation, and apoptosis contributing to cognitive decline.. Persea americana, commonly known as Avocado, is a large berry-shaped fruit which is enriched with lutein and is helpful in improving brain functions. Lutein is a carotenoid which is commonly found in fruits and vegetables that accumulates in the blood, eye and brain. It may act as an antioxidant. So, it can be hypothesized that Persea americana might have neuroprotective properties and it might help in preventing brain aging. For this purpose, aging in rats was induced by D-galactose via oral administration for 6 weeks followed by supplementation of Avocado pulp and seed extract. After induction and treatment, neurobehavioral analysis was conducted followed by decapitation and brain dissection to collect hippocampus and cortex for neurochemical and histological analysis. Results showed that compared to avocado seed extract, avocado pulp is effective in improving spatial memory, muscle strength, anxiety, locomotion, depression and episodic memory and cognitive performance which is further validated by histopathological examination and alteration in levels of GABA and Glutamate. Thus, avocado pulp is found to be effective in preventing D-galactose-induced brain aging and can be suggested as a anti-aging supplement to improve neurological functioning.

Biofilm Formation Potential: Comparison Among Oral Microbial Isolates

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Biofilm formation is one of the important characteristics of oral microbiome. Apart from Smoking Tobacco Products (STPs), Chewable Tobacco Products (CTPs) consumption have also been followed in regular pattern specially in Pakistan. These tobacco products not only harm the Oral Cavity but also damage other systems of human body. The microbial contaminants of CTPs play a major role in altering the normal physiology of oral cavity i.e. formation of microbial contact layer called Biofilm. The present study was conducted to have a comparative analysis of biofilm formation by oral isolates of Chewable Tobacco Consumers (CTCs) and Non-Chewable Tobacco Consumers (NCTCs). Convenient sampling technique was followed with full consent. 10 samples from NCTCs were collected (as Control) and 20 from CTCs divided as 10 from gutka consumers (test-T1) and 10 are those who use gutka with other chewable tobacco products (test-T2). Biofilm screening was performed using both qualitative & quantitative analysis. Results revealed presence of large number of G-ve bacterial species in the oral cavity of CTCs group T1, compared to control (C) group having NCTCs giving approximately same number of G+ve and G-ve bacterial species. Fungal isolates were absent in group T1 whereas group T2 showed presence of mold and yeast fungi, on the other hand C group contained only yeast isolates. When we compare the biofilm formation potential of test-T1 & T2 and C groups, interestingly C group showed better biofilm formation, compare to the test groups and found to be weaker in this character. Reduced biofilm formation in test groups might be due to the abundance of G-ve bacterial isolates that may suppressed the growth of G+ve bacterial isolates, as G+ve bacterial species found in oral cavity are considered as the good biofilm formers. This study may be concluded that the difference in the biofilm formation of NCTCs and CTCs might be due to the inhibition of normal flora which could be further deciphered at larger scale using multiple parameters.

Key words: Biofilm, Chewable Tobacco Products

Diversity and enzymatic activity of fungi isolated from mangroves of Makran coast Balochistan

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The aim of this study was to isolate fungi from Makran coast of Balochistan and to screened them for their ability to hydrolyzed starch and pectin for the production of amylase and pectinase respectively. In this present investigation, mud, stem, leaves, bud and muddy water mangrove samples were collected from different locations of Makran coast of Balochistan and were preserved in vials containing glycerol. By streaking the obtained mangrove samples on yeast peptone dextrose (YPD) plates media, seventy three different fungal strains out of hundred mangroves samples were revived. Mixed or different colonies were allowed to grow on separate YPD plates. The pure fungal cultures were identified on the basis of microscopic examination. All the strains were accessed for the production of extracellular enzymes amylase and pectinase by plate assay method using starch and pectin as the substrate respectively. The development of clear zone was regarded as positive result for the enzyme activity of the respective mold. The result of present study revealed that 73% fungal strains were revived out of hundred preserved samples and by separating mixed or different fungal colonies, total pure ninety eight different fungal strains were obtained. The identified strains were belong to *Aspergillus, Cladophialophora, Cladosporium, Fusarium, Gliocladium, Mucor, Penicillium, Rhizopus, Trichothecium* species. Out of ninety eight pure fungal strains, 71% isolates were able to hydrolyzed the starch and showed amylase production while 67% isolates were able to produced significant amount of pectinase from the hydrolysis of pectin. On the basis of this study, it has been concluded that variety of fungal species are present in marine environment and these fungal species are capable of producing industrially important enzymes, amylase and pectinase that are widely used in textile, paper, pulp, food and in many other industries.

Keywords : Mangroves, Molds, Microscopic identification, Amylase, Pectinase.

Role of microbial metabolites on biofilm formation by Candida species

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Candida is one of the peaceful microorganism which lives as normal commensal in the host body. But sometimes their overgrowth may cause severe infections in different parts of the body. Most of these infections are associated with their ability of biofilm formation which is an important virulence factor which contributes in pathogenicity of *Candida.* Biofilms of *Candida* species can be influenced by growth of other microorganisms in the same environment.. In order to investigate the role of microbial metabolites in the inhibition or enhancement of biofilm formation by *Candida* species, this study was carried out. The effect of metabolites produced by bacteria strains *Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and fungal strains *Aspergillus flavus* and *Mucor spp.* on biofilm formation by fifty strains of *Candida* species (*Candida albicans, Candida tropicalis, Candida glabrata, Candida krusei* and *Candida rugosa*) was investigated. The cell free supernatant (CFS) of the bacterial and fungal strains were prepared and their effect on biofilm formation by *Candida* species was detected by using tube staining assay. The excellent inhibitory effect on biofilms of *Candida* was found in the presence of metabolites produced by *M.luteus* and *Mucor* sp. However, the bacterial metabolites of *S.aureus, P.mirabilis* and *P.aeruginosa* showed an excellent synergistic effect on biofilm formation and strong biofilms were formed by *Candida* species in their presence. Metabolites of *B.subtilis* and *A.flavus* were found to have no effect on biofilm formation by most of *Candida* species. This concludes the potential of different bacterial and fungal metabolites in inhibition or enhancement of biofilm formation by *Candida* species and further investigation in this regard may help in future for designing any strategy to control biofilm formation by *Candida* species.

Key words: Candida, Biofilm, Metabolites, *C.albicans, C.tropicalis*

Stereoselective Hydroxylation of Norethisterone by using *Aspergillus niger* and *Pencillium citrinum*

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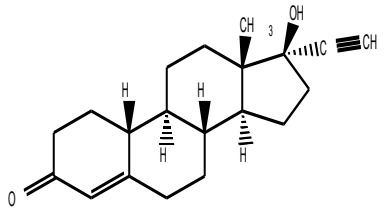
Abstract

Biotransformation has been extensively employed for the synthesis of fine chemicals by incubation with enzymes, plants, animals and microorganisms (fungi and bacteria) for years, which may be difficult to synthesize by chemical methods [1]. It is also known a well-established method for the large scale manufacturing of steroidal drugs [2].

Fungal transformation of norethisterone (**1**), a famous drug is studied in the present work. Norethisterone (**1**) yielded two metabolites **2** and **3** by incubation with *Aspergillus niger* and *Pencillium citrinum*. This is the first report of their production from biotransformation of norethisterone (**1**) using these fungi.

The structure of transformed products **2** and **3** were elucidated by modern spectroscopic methods including IR, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR techniques.



Norethisterone (1)

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BIOPROCESSING of AGRICULTURAL WASTES for VALUE ADDED BACTERIAL AMYLASE PRODUCT

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ABSTRACT

Potential Amylase enzymes have many industrial applications that are found in biological Sources like animal, plants and microorganisms. Fungi and bacteria hold tremendous potential to produce the α-Amylases using agriculture by-products under solid state fermentation (SSF). Agro-industrial residues such as rice bran, wheat bran, sugar cane burgesses, corn leaf, barley, orange peel, wheat straw, rice straw are abundant and cheapest carbon source. SSF using agro- industrial residues is currently used in a range of applications including classical applications such as antibiotics production, enzymes, composting, bio-surfactants and biofuel production. Microbial α-amylases have several applications in paper, food, pharmaceutical, detergent, and textile industries. The enzyme α-amylase is meritorious due to their properties such as thermostability, Ca 2+ -independent pH stability and pH profile which play important role in the development of bioprocess of different products. This review is focused on physiochemical properties of the bacterial α-amylases fermentation, structural and functional aspects of agro industrial residues and by products for α-amylase production.

Key words: agriculture by-products, agriculture residue, α-amylase, Solid state Fermentation.

BIOLOGICAL PRETREATMENT OF SUGARCANE BAGASSE FOR THE PRODUCTION OF LACCASE

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ABSTRACT

Sugarcane is one of the important crops in Pakistan which is mainly used to extract sugar in industries and generate huge quantities of waste material, bagasse. Economic utilization of sugarcane bagasse and other agro-industrial wastes offers prospects for the foundation of biotechnological indus-tries in our country. Microorganisms including bacteria and fungi are considered the suitable candidates to utilize such substrates; however, the presence of lignin limits the microbial growth. Some fungal species especially white-rot fungi have previously been reported to play role in the process of delignification. During their growth, these fungi produce an industrially important enzyme, laccase. Among the white-rot fungi, *Trametes* spp. are considered as the promising ligninolytic agents. Keeping in view this aspect, this study was designed to produce laccase by using sugarcane bagasse (SB) as a substrate. For this purpose sugarcane bagasse was collected from the local seller as well as from the sugar industry. *Trametes pubescens* MB89 culture available in the lab at the Department of Microbiology was used to produce laccase enzyme. The fungal culture was then transferred into the flask containing the sugarcane bagasse. Solid-state fermentation (SSF) was carried out. After incubation, the laccase enzyme units were calculated at 420nm by using 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS). The amount of the substrate in the assay mixture varied from 10 µL/ml, 20 µL/ml, 30 µL/ml, 40 µL/ml to 50 µL/ml that led to the enzyme activity as 17,58 Uml^{-1}, 18.53 Uml^{-1}, 17.52 Uml^{-1}, 11.54 Uml^{-1} and 19.33Uml^{-1}. The factors affecting SSF of SB were screened using a statistical tool, Plackett-Burman design. The study revealed potential of the strain MB89 to produce laccase under SSF of SB.

Key Words: Laccase; Sugarcane bagasse; Solid state fermentation; *Trametes pubescens*

Anti-Parkinson activity of crude Scorpion Venom *(Buthus sindicus)*

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Background: Parkinson disease (PD) is common neurological disorder characterized by dopaminergic neuronal damage in nigrostriatal pathway, manifested as motor function and non-motor function deformities. There are number of conventional therapeutic options available but are associated with severe side effects and have limited symptomatic relief. This increases the demand of finding new therapeutic options. Natural resources have great significance for drug discovery as always. Scorpion venom has been emerging as new resource to have therapeutic potential from a cocktail of variety of peptides, enzymes, chemicals and proteins. *Buthus sindicus* is Pakistani yellow scorpion that belongs to medically important family Buthidae. **Objective:** The study was designed to assess the anti-Parkinson activity of scorpion venom *Buthus sindicus* at different doses *on* haloperidol-induced mouse model of PD. **Methodology:** Male NMRI mice weighing 25-30g were used and divided into 04 groups (n=5). Group I was treated as normal control, group II was negative control treated with haloperidol (1mg/kg *i.p*) only, group III was positive control treated with haloperidol and conventional drug (Levodopa plus carbidopa 100mg+10mg/kg *p.o*) and group IV was treated with scorpion venom (0.01 µg/kg *i.p*). All the animals were subjected to be drug treatment for 07 days. Evaluation was done by behavioral assessment through catalepsy bar test and block test, wire grip strength test, open field test and forced swim test. Toxicity profile was also checked through acute and subacute treatment of venom. **Results:** Our results show that scorpion venom decreases the time of animal staying onto the bar improving the cataleptic behavior. Similarly, it has shown no signs of toxicity on tested dose exhibiting anti-Parkinson activity. These are the preliminary results first time reported in Pakistani Buthus *sindicus*. We also suggest that scorpion venom could serve as lead resource to search potential anti-Parkinson drug molecules. However, further studies are required for characterization and fractionation of venom to identify the peptide responsible of showing anti-Parkinson effect.

Keywords*:* Scorpion, *Buthus sindicus,* Anti-Parkinson, haloperidol, catalepsy

Association of Dopamine Receptor Type 2 Gene Variants with Schizophrenia in Pakistani Population: A First Report

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Schizophrenia is a chronic recurring mental illness which has affected 1% population worldwide. It is characterized by hallucinations, delusion, susceptibility and isolation. Dopamine receptor type 2 (*DRD2*) is the major target while treating schizophrenia. Polymorphisms in DRD2, exon7 rs1801028 and rs6277 found associated with schizophrenia in different populations worldwide. However, ethnic discrepancies exist. These polymorphisms have not yet been studied in Pakistani population. Therefore, this study aimed to explore the association of these polymorphisms in Pakistani schizophrenic patients. Around 100 schizophrenia cases and 100 healthy controls were recruited for the study. Severity status was determined using PANSS score. Genomic DNA was extracted from peripheral blood. Region of interest was amplified using PCR. PCR products were then sequenced, and aligned using MEGA X software against the reference sequence from NCBI. Statistical analysis was done using SPSS version 20. Results show no significant difference in genotype or allele frequency for any of the polymorphism when schizophrenia patients were compared with control group (P>0.05). No association of PANSS score was found with any of the above mentioned polymorphisms (P>0.05). Interestingly a novel mutation at chr11:113412805 (C>A) was spotted with statistically significant difference among cases and controls (P=0.001). This mutation resulted in the substitution of amino acid proline with threonine as confirmed by MEGA protein translate tool. It is exciting to report that frequency of homozygous wild type genotype (CC) was higher in schizophrenia patients (67%) than in control (43%) group. In contrast heterozygous genotype (CA) was higher in controls than in cases (57%, 33% respectively). While, frequency of mutated allele (A) of this novel mutation was comparatively higher in controls than cases (28.5%, 16.5% respectively). Our data showed that A allele might have protective effect and that C allele may be associated with schizophrenia, being found in higher frequency in cases (83.5%) than in control group (71.5%). Thus homozygous AA genotype may confer a protective effect to schizophrenia in our population. The actual mechanism conferring this protective effect is not clear. Future research in this respect may reveal the mystery. Apparent lack of association of other polymorphism with schizophrenia may be due to ethnic differences. Multicenter replication studies with large sample size are encouraged.

Skimmia laureola mediated silver nanoparticles (SKL-AgNPs): An innovative approach for catalytic reduction of congo red dye

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Congo red (CR) [1-naphthalene sulfonic acid, 3,30-(4,40-biphenylenebis (azo)) bis (4-amino)disodium salt] is a benzidine-based anionic di-azo dye. It is extensively use in textile, paper, rubber and plastic industries and cause serious ecological damage to the environment if they are discharged without proper action. Its toxic behavior towards several organisms is the key evidence for its characteristics carcinogenicity and mutagenicity. Its complex aromatic structure enhances its physicochemical, thermal and optical stability which render it to be non-biodegradable. In this scenario an immediate action is required to develop such method which remove the dye molecules from the industrial influent at low cost. Metal nanoparticles have relatively large surface-to-volume ratio which tends to enhance their catalytic activity towards degradation of organic dyes. In the current research work synthesis of *Skimmia laureola* commonly known as Nazar Panra mediated silver nanoparticles (SKL-AgNPs) is carried out using leaf extract of *Skimmia laureola* (SKL) as capping and reducing agent. These highly efficient SKL-AgNPs are ecofriendly and cost effective. The reduction of congo red was done by $NaBH_4$. The reduction capability of $NaBH_4$ was observed to be enhanced by these SKL-AgNPs which was continuously monitored by UV-Visible spectrophotometer. The results showed that these nanoparticles exhibit a high catalytic activity in the reduction of congo red dye.

Keywords: *Skimmia laureola,* Silver nanoparticles, Catalytic reduction, Congo red

Fe-Cr-Ni and Cr-Co-Zn Nanocomposites and their Microbiological Activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Nanotechnology plays a significant role in our society because it deals with matter at the scale of 1 billionth of a meter. Nanoparticles have some unique characters including physical and chemical properties which intensify their applications in nanotechnology. This research discusses the synthesis, characterization and antibacterial applications of nanocomposites. The aim of the study is to evaluate the antibacterial potential of chemically synthesized metal oxide nanocomposites and its applications. The antimicrobial potential of nanocomposites was checked against gram positive and gram negative bacteria, yeast and mold fungi by agar well diffusion method. A total of 10 samples of nanocomposites were screened against 10 bacterial and 7 fungal strains. Out of which the maximum zones of inhibition was produced by Nanocomposites Fe-Cr-Ni and Cr-Co-Zn against *S. aureus* and *P. aeruginosa.* The bacteriostatic or bactericidal effects of these samples were checked in different concentrations by optical density method. The antibacterial effect of Fe-Cr-Ni and Cr-Co-Zn treated leather samples was assessed on *S. aureus* or *P. aeruginosa* by agar diffusion method and optical density method. The results showed effective antibacterial effect of nanocomposites treated leather samples against both of the bacterial strains. In future, this research would be helpful in the footwear industry by reducing the unpleasant smell produced by bacteria.

Molecular Characterization of Drug Resistance in Uropathogenic *Escherichia coli* from Karachi, Pakistan

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Multidrug resistance has become a major public health issue associated with considerable morbidity and motility. Therefore, the prospects of study are to investigate molecular mechanism of quinolone resistance, CTX-M genotypes and clonal relationship in uropathogenic *E.coli.*

UPEC showing resistance to 3rd generation cephalosporins were evaluated for production of β-lactamase by double disk synergy test and plasmid profile analysis. Also, MICs for cefotaxime and ciprofloxacin were determined by agar dilution technique. The presence of the *bla* SHV, *bla* TEM and *bla* CTX-M genes was assessed by PCR method and *bla* CTX-M producing strains were further genotyped into phylogenetic groups (CTX-M -1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) and sequenced while, the plasmid mediated quinolone resistance genes *qnrA*, *qnrB* and *qnrS* were determined by multiplex PCR and chromosomal resistance was assessed by amplifying and sequencing of *gyrA* and *parC* genes for investigating the mutations and finally genetic relatedness among *E.coli* was carried out by ERIC PCR.

Of the 74 uropathogens, 50 isolates representing 68% were *E.coli* that showed 100% resistance to fluoroquinolone and high resistance to third generation Cephalosporins, (Cefotaxime and Ceftriaxone) i.e. 90%, MICs of Ciprofloxacin against 50 isolates of *E. coli* were >1024 µg/ml, MICs of Cefotaxime against 46 isolates of *E. coli* were >128 µg/ml. Out of 50 *E.coli* 33 (66%) were ESBL by phenotypic method. MDR *E. coli* strains possess multiple plasmids of variable banding pattern. The prevalence of *bla* CTX-M, *bla* TEM and *bla* SHV genes among these isolates were 86%, 62% and 6% respectively, found in single or in combination (s). Genotypic analysis of CTX-M has revealed that 40 (80%) *E. coli* belong to CTX-M G1 and 3 (6%) to CTX-M G9 while CTX-M G2 and CTX-M G 8/25 were not detected in the isolates studied. Sequencing of 10 selected isolates of *E.coli* with CTX-M G1 showed sequence similarity with CTX-M 15. Plasmid mediated quinolone resistance genes were identified in 31 isolates, including *qnrB* in 9 (18%) and *qnrS* in 8 (16%) and *qnrB* in combination with *qnrS* in 14 (28%) while, *qnrA* was not detected. Several alterations were detected in *gyrA* and *parC* genes in QRDRs and ERIC-PCR revealed genetic diversity among UPEC.

High fluoroquinolone resistance and growing prevalence of CTX-M-15 and TEM β-lactamases in *E. coli* emphasizes the need for antibiotic stewardship, epidemiological monitoring and warrant a review in quinolone and cephalosporin based empirical treatment of UTIs.

Physicochemical and microbiological assessment of the ground water resources of Karachi city, Pakistan

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Due to industrialization and over population, surface water resources are out of reach from many people so consumption of ground water is the only choice to overcome the water scarcity. Naturally, ground water is one of a significant and potable water resource but some geographical conditions and anthropogenic activities deteriorate the water quality and make it objectionable for drinking. This study was conducted to evaluate the ground water quality of Karachi, Pakistan. For this, forty-two ground water samples were collected from different towns of all six districts of Karachi and analysed their physicochemical and microbiological characteristics then compared it with both international (WHO) and national (SEQS) drinking water standards. In the physical assessment, pH, temperature, turbidity and taste of ground water were analysed whereas in the chemical assessment concentration of total dissolved solids (TDS), total hardness as calcium carbonate, sodium and chloride were assessed. Moreover, heavy metals (arsenic, lead, cadmium, zinc, copper and iron) were also assessed by using atomic absorbtion spectrophotometer. Microbiological assessment of ground water was also performed in present study, in which number of heterotrophs (aerobic), coliforms, fecal coliforms and fungal contamination were analyzed. Results of this study declared that all physical parameters were exist in the permissible limit of both drinking water standards whereas, chemical assessment depicted that chemical constituents like TDS, total hardness as $CaCO_3$, chloride, arsenic, lead and cadmium concentrations were high in the ground water samples of West, South, Central and Malir districts of Karachi. However, concentration of sodium, zinc, copper and iron were present within the prescribed limit of both standards. Microbiological assessment of present study describe that all ground water samples have coliforms while only in the samples of East and Malir districts of Karachi, fecal coliforms were not observed. Whereas, heterotrophic (aerobes) and fungal contamination was also observed in the ground water samples of Karachi. This deteriorated ground water quality of Karachi can be improved by maintenance of proper sanitary conditions of the communities and implementation of water treatments, otherwise consumption of such water may develop serious health related consequences in the consumers.

Keywords: Ground water, Potable, Arsenic, Lead, Cadmium

EFFECTIVENESS OF BUSPIRONE IN AMELIORATING NICOTINE-INDUCED ADDICTION AND RELATED NEUROLOGICAL DEFICITS

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Buspirone has a recognized effectiveness for the treatment of anxiety, in addition to it does not have hypnotic, muscle relaxant and anticonvulsant properties so termed as anxio-selective drug. It also has been reported that it shows significant results in reducing drug addiction such as cannabis and opium, but effects of buspirone on nicotine addiction have not been assessed. Thus, present study purposed to evaluate the impact of administration of buspirone on nicotine addiction induced neurological deficits in healthy young rats. Two major groups control, and nicotine were designed that were further subdivided in two sub groups, the buspirone group received buspirone intra-peritoneally at the measured quantity of 1mg/ml/Kg, while the control group received only 0.9% saline, the buspirone plus nicotine group received nicotine addiction along with the administration of buspirone while, the buspirone group received buspirone along with administration of saline. The whole treatment was continued for 14 consecutive days. Body weight and food intake were assessed at the regular interval of instance and the behavior examination that was consist of evaluation of cognitive enhancement (through novel object recognition and Morris Water Maze test), anxiety-like behavior (through elevated plus maze test), Depression (through Forced swim test) and locomotor activity (through Open field and Kondziela's inverted screen); were performed. After behavioral analysis, the serum samples of rats were collected for evaluation of total protein, creatinine, triglycerides, glucose, urea and cholesterol to conduct biochemical analysis. The results revealed that the administration of buspirone leads to significant improvement in cognitive function, reduction of depression and anxiety-like symptoms and improvement in locomotor activity as well as reduction in biochemical deficits caused by nicotine addiction was also found as enhanced protein and glucose levels in plasma of buspirone treated rats was observed. Hence, as buspirone is beneficial in improving neurobehavioral, and biochemical deficits in addicted rats so it can be suggested in future as a safer therapeutic and preventive approach for ameliorating drug addiction and its reinforcing effects related disturbances.

Nicotine ; buspirone; addiction; biochemical analysis; neurological deficits.

Antagonistic potential of indigenously isolated *Bacillus sp.SSM08* against phytopathogenic *Fusarium* species

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Fungal phytopathogens are a foremost hazard to the stability of agriculture and ecosystem. The use of chemical fungicide over the years has resulted in emergence of resistance in pathogenic strains, thereby necessitating the development of effective and environmental friendly approaches. The natural antagonistic interactions among different microbial populations have been exploited as an eco-friendly move towards management of resistance in fungal pathogens.This study was initiated as one of the approach to assess inhibitory potential of some microbial metabolites towards phytopathogens. In this regard, morphologically distinct bacterial strains were isolated from rhizospheric soils collected from the garden area of Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan by utilizing spread plate technique. The isolated strains were screened by using agar well diffusion method for their antifungal potential against *Fusarium species*, the well-known seed-borne, seed-transmitted, soil-borne and soil-transient plant pathogens. Among all the strains, the cell suspension and the cell-free supernatant of *Bacillus* sp.SSM 08 (identified conventionally by morphological and biochemical characters) exhibited significant antifungal activity against *Fusarium sp.* as evidenced from zones of inhibition. To maximize the production of antifungal metabolite by this *Bacillus sp.,* optimization of various physicochemical parameters like strong agitation, media composition, temperature and pH were also carried out. Biocontrol potential of this bacterial isolate against strains of plant pathogen *Fusarium* species has opened up chances for its applicability as promising biocontrol agent in near future.

Key words: Phytopathogens, biocontrol, Bacillus, Fusarium

Identification of Differentially Expressed Genes (DEGs) Associated With Progression and Prognosis in Patients with Malignant Melanoma

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The primary malignant melanoma is type of a skin cancer which starts from skin and then invades in different organs. The present study was accomplished to find out DEGs for better understanding the mechanism of melanoma. In silico analysis was carried out to identify DEGs from mRNA dataset (GSE GSE123686). This dataset includes 12 samples 3 from healthy and 9 from disease patients. Biological roles of DEGs were further analyzed by gene ontology, pathway enrichment, protein-protein interaction (PPI) network. A total of 250 DEGs were identified, among them 139 were up & 61 down regulated. DAVID tool predicted the involvement of DEGs in 17 biological processes, 5 molecular functions and 9 cellular components. KEGG pathway enrichment predicted the involvement of DEGs in ATP-dependent RNA helicase activity, RNA methyl transferase activity, positive regulation of actin filament bundle assembly, glucocorticoid receptor signaling pathway, establishment of spindle localization, negative regulation of oxidative stress-induced cell death, spliceosomal snRNP complex, u2-type precatalytic spliceosome and u4/u6 x u6 tri-snRNP complex. PPI network and cytoscape tools predicted a total of 198 nodes and 686 edges among DEGs. The DEGs with highest degree of interaction (HUB Gene) includes MED1, MED17, MED13L, MED16, MED13, MED12, MED15, MED14, MED11 and MED10. Furthermore, 179 DEGs from our GSE GSE123686 and 222 DEGs of GSE GSE83922 were compared by using venny online tool and venn diagram was constructed which identified "JUN" is common in both melanoma samples. Our results contribute to the exploration of further basic and clinical research of melanoma.

Keywords

Melanoma, DEGs, Bioinformatics analysis, DAVID

Class 1 & Class 2 Integrons in Clinically Isolated Multi Drug Resistant *Pseudomonas aeruginosa*

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Integrons are mobile genetic elements and one of the major players responsible to disseminate the antibiotic resistance among bacteria. *Pseudomonas aeruginosa* is one of the leading causative agents of severe nosocomial infections, shows remarkable resistance against a range of antimicrobial agents and raised as multidrug resistant super bug. Hence aim of the present study was to investigate the presence of class1 and class2 Integron genes (*int1 and int2*) among clinically isolated multi drug resistant (MDR) *Pseudomonas aeruginosa*. A total of seventy clinical isolates of MDR Pseudomonas strains collected from different clinical sources such as Blood, Urine, Tissue, Tracheal aspirates and Pus samples mostly of Burns patients were screened for the occurrence of Class1 and Class2 Integrons. Initially the organisms were identified as *P. aeruginosa* by standard biochemical tests while sensitivity profiles were established by disk diffusion method using a panel of 11 different antibiotics representing 7 different classes. Each of the isolate was screened after DNA extraction for Class 1 and 2 Integron gene by PCR amplification using specific set of primers. Further the Integron positive strains were identified by 16S rDNA gene sequencing. Results revealed that out of 70 tested MDR isolates 29 (41.4%) *Pseudomonas* strains carried Class 1 Integron and only 12 (17%) MDR strains harbored Class 2 integron genes. Interestingly all the class 2 integron positive strains were also positive for Class 1. Distribution of both Integrons was highest among burns patient. Antibiotic susceptibility test showed that Colistin was the most effective antibiotic with 3.4% resistance while higher resistance patterns were observed in Cefepime (95.7%) among isolated MDR *Pseudomonas* strains. While upon 16SrDNA based identification majority of the Integron positive strains were identified as *Pseudomonas aeruginosa*. In conclusion prevalence of Class1 and Class2 Integrons among indigenous MDR isolates is an alarming situation, indicating the possible role of these mobile elements in dissemination of antibiotic resistance among different bacterial pathogens.

Key Words: Integron, Pseudomonas, Antibiotic resistance.

In Silico Approach to Identify nsSNP of ADRB2 Gene

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ADRB2 is G-protein-coupled receptors which comprises of the biggest super-family of signaling molecules. They consist of an extracellular amino terminus, seven transmembrane-spanning α helical regions, three extracellular loops, three intracellular loops, and a C-terminal intracellular tail. They carry out signaling via coupling to guanine nucleotide binding proteins (G-proteins). ADRB2 is localized at chromosome 5q32 and encoded 413 amino acids.

Current study was planned to find out SNPs ADRB2 gene and their effect on gene function and structure. A total of 978 SNPs were retrieved from Ensembl SNP database. These included 409 in the 5' prime UTR variants, 197 in the 3' prime UTR variants, and 130 synonymous variants, 225 missense variants, 10 frame -shift variants, 3 stop gained, 2 start lost and 2 in-frame deletion. During present work only missense SNPs were characterized by bioinformatics tools.

Among 228 nsSNPs, 15 were predicted to be deleterious (rs140420820, rs142595411, rs201318801, rs143912954, rs201257377, rs201323763, rs41320345, rs201644897, rs373763314, rs374211656, rs371880712, rs374596758, rs370652553, rs144618094, and rs151019714) from SIFT analysis. These 15 nsSNPs were further analyzed by 8 online tools and only 3SNPs (rs140420820 (M82K), rs201318801 (F290S) and rs201644897 (S329I)) were predicted to be the most deleterious, as supported by nine of the nine in silico tools. To understand to structural changes, mutant models of 3 deleterious mutations were generated.

Protein-protein interaction analysis showed that 10 different proteins were interacting with native ADBR2. Whereas M82K loss the interaction with 2 proteins. As M82 is involved in ligand binding site so the mutation at this site may also effect on protein function.

Keywords

nsSNP, ADRB2, In silico analysis

Electrochemical Methodology of NSAID's Interaction with Steroidal Drug Dexamethasone

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Almost more than 33 million people in all over the world taking daily nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are prescribed in combination with other drugs for the treatment of diabetic, hypertension, cancer treatment etc. They are also taken in combination with steroidal drugs. The co-administration of multiple drugs may result in drug-drug interactions (DDIs) capable to alter the efficacy and toxicity of the individual drug. In this current study development of an electrochemical method Cyclic Voltammetry (CV) for the analysis of Naproxen Sodium and Piroxicam by varying different parameters like supporting electrolytes, solvents, pH, electrodes and scan rate to achieve the optimum conditions. The recommended method is diffusion controlled. The experimental diffusion coefficients were 3.755×10^{-5} cm^2/s and 2.188×10^{-7} cm^2/s for Naproxen Sodium and Piroxicam respectively. The proposed method was validated according to ICH guide lines. The % recovery of the proposed method was 99.64 and 99.08 for Piroxicam and Naproxen Sodium respectively. After the successful method development; it was applied on the interaction studies of both NSAIDs with a well-known steroid Dexamethasone. This research work first time reports drug-drug interactions studied by the cyclic voltammetry. FT-IR spectroscopy was performed for further confirmation of the interactions between the two molecules and their interaction sites. Dominating peaks of all four NSAIDs effected after interaction with Dexamethasone. The reaction site of Dexamethasone is undoubtedly its O-H stretching, as a very significant absorption peak at 3522 cm^{-1} completely disappeared in case of Piroxicam and Naproxen Sodium and for Ibuprofen and Flurbiprofen its intensity decreases.

Keywords: NSAIDs, Cyclic Voltammetry (CV), FTIR, UV-Visible Spectroscopy

Role of oxidative stress and effect of antioxidants in the progression of Osteoarthritis

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Globally, more than 150 musculoskeletal conditions have been diagnosed that distress the locomotor system. Osteoarthritis, rheumatoid arthritis, fragility fractures, back and neck pain are amongst the most disabling musculoskeletal conditions. Osteoarthritis (OA) is a degenerative joint disease with major socio-economic impact. In Pakistan, It has been reported that 25% rural and 28% urban population suffered from knee osteoarthritis with the ratio of 1:4 male to female respectively. Diagnosis and treatment at initial phase of infirmity are still hot area of research. However non curative interventions such as exercise and psychological remedies might be helpful to manage the sicknesses. The risk factors for developing OA are mechanical stress, joint injury, age, gender**,** obesity, genetics and oxidative stres.. Mean of access through which reactive oxygen species (ROS) contribute to OA pathology is still unknown and there is a need to study the role of antioxidants in OA. The rationale for the present study is to explore the association of oxidative stress as a contributing factor for the progression of disease. Focus of the current study is the determination of single nucleotide polymorphism in gene of antioxidant enzyme along with the level of Malondialdehyde (MDA). We have observed a possible association between L55M polymorphism and osteoarthritis. Level of MDA, a potential biomarker for lipid peroxidation and extent of protein carbonylation as a marker of protein damage measured and found increased in lipid peroxidation product and carbonylation content in OA patients as compared to healthy individuals.

Keywords: Osteoarthritis – oxidative stress – antioxidants – gene polymorphism

Amylase Production by Bacillus Species Using Agro-Industrial Residues as a Substrate

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There are six major classes of enzymes Oxidoreductases, Transferases, Hydrolyses, Lyases, Isomerases and Ligases. Amylases are classified as Endoamylases or α amylae, Exoamylases or ß-amylase, Pectinases or Pullulanase and Cyclodextrin or glycosyltransferases. The cleavage of 1, 4-linkages of starch inner region Agriculture Waste Management System recommends 3R approach, (reduce, reuse and recycle) for the agricultural waste management. Agro-industrial wastes contains high contents of carbon, which is useful for bacterial amylase production due to short period, value added production, stability in uttermost conditions, isolation in bulk quantity, and their end-compounds are harmless and very controllable. Amylase production in Solid Stand Fermentation SSF is restricted to genus Bacillus, i.e *B. megaterium*, *B.mesentericus*, *B. vulgarus*, *B. subtilis*,*B.licheniformis* and *B. polymyxia* while thermo stable amylases of *Bacillus stearothermophilus* or *Bacillus licheniformis* are being used in starch processing industries. Agro-industrial residues rice brane, wheat brane, Sugarcane bagass, Banana waste and Cotton stalk were practice as low cost carbon source for bacterial (bacillus) amylase production. The major fermentation factors which effect on the amylase production are as follows Incubation period, pH, nitrogen sources, temperature, phosphate, carbon source, surfactant, moisture contents in both Smf and SSF media and metal ions.

Key words: Amylase, Agro industrial residues, Bacillus.

Teratogenic and Carcinogen Modelling of Bispehnol-A in *Drosophila melanogaster*

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Bisphenol-A is a compound extensively used in the manufacturing of plastics. In recent times, several studies have pointed its toxicological effects on human health. *D.melanogaster* is a model organism for studying genetics, developmental biology and human diseases. The primary aim of the present investigation is to explore the effect of Bisphenol-A, a compound used mainly in the plastic industry, on the brain development in *D. melanogaster* fruit fly. Briefly, outbred D. melanogaster flies inbred line were exposed to Bisphenol-A by dividing the flies into five sets: control group, 1mM, 2mM, 4mM and 10mM Bisphenol-A in consistent and incidental manner. At the end of the experiment, fecundity and longevity of the flies were assessed. Flies from each groups were compared for morphological traits including, body size, regional size, eye size and shape and wings size and shape. Three flies each from the parental group and offspring were subjected to histological investigation and size of the brain were measured and compared. Exposure of the Bisphenol-A resulted in the noticeable change in various traits compared to the control set (standard cornmeal medium). Significant delay was observed in the purpariation of the flies, which points to the dysregulation of ecdysone pathway. System network analysis of orthologous genes in humans culminates to the molecular pathways that underpin oncogenesis. Histological analysis also indicates the noticeable neurodegeneration and aberration in direct flight muscle anatomy of the flies. Finally, fecundity, sexual bias in F1 generation and assessment of still birth by scanning electron microscopy points to the teratogenic potential of the BPA.

Key words: Bisphenol-A, Brain, Drosophila, fecundity, longevity

Molecular Modeling and Structural Polymorphism of Bovine Dehryogenase Reductase

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Dehyrdogease Reductase (DHR) is a oxidoreductase catalytic protein mainly involved in cellular balance of calcium. Recently the protein has been linked with the development of horn in *Bovidae* and/or cetaartiodactyla. Since in artiodactyla, horns exist in different forms and shape, it is potentially possible that structural differences in the enzymes may lead to the different functional consequences which in turn underpin phenotypic appearance of different forms of horns. To explore this possibility, full length molecular models of the DHR of bovine was developed by iterative threading. After structural and thermodynamic refinement, the model was used as template to develop the structures of other mammalian orthologues by comparative modelling. Structural and thermodynamic assessment scores are well within reliable limit denoting the structural reliability of the model. The protein mainly comprises hydroxysteroid dehydrogenase type 1 domain (83-344 aa) which adopts a classical structure reserved for the respective domain, containing 6 antiparallel β sheets surrounded by 6 helices. The structure form three main pockets, of these the largest funnel shape cavity holds functionally important residues. Comparison with orthologous structures demonstrate noticeable deviation in the Cα backbone structure and dimensions of the cavity which may lead to the functional divergence of the protein.

Key words: Bovidae, DHR, Evolution, Molecular Modelling.

Evolution of Whole Genomic Synteny of *Flaviviridae*

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Family *flaviviridae* includes RNA containing viruses like Dengue virus, zika virus, yellow fever virus and hepatitis C virus. The viruses of the family typically encode a polyprotein which later cleaved into structural and functional subunits. The present study is designed to explore the physical map of genomes of *flaviviridae*. Briefly, phylogenetic reconstruction was carried out on the basis of poly protein of viruses using NJ method with 1000 bootstrap replicates. The tree then aligned with the genomic map of the viruses as retrieved from NCBI and/or Viral Zone. The data shows noticeable gain and loss of genes such as VP1, 2 and 3 in different lineages of the viruses. Variations in the order and span of the genes were also observed. This point to the substantial recombination events followed by extensive genomic rearrangement during the course of genome evolution in *flaviviridae*.

Key words: Genome, Polyprotein, *flaviviridae*, Evolution

Molecular Structure of Non-Structural Protein 1 (NSP1) of Guaico Culex Virus

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Guaico Culex Virus (GCV) is a recently discovered RNA containing virus of family *Flaviviridae*. Like other members of the family, the virus encodes a non-structural protein, NSP1 which is responsible for RNA capping during viral propagation. To date the molecular structure of the protein is not known. In this study we have developed the full length molecular model of NSP1 of GCV by homology modelling using atomic coordinate of 5ZQK. Ramachandran plot values and ProSA Z score are well within acceptable limit suggesting structural reliability of the model. Structurally, the protein comprises of two main domains namely, Fts J like methyl transferase domain and RNA directed RNA polymerase domain. Fts J like methyl transferase domain (95-267 aa) adopts a structural unit comprises three pairs of antiparallel β sheets with three helices. Of these, two of the helices are parallel to the sheets whereas one is oriented obliquely. The polymerase domain is relatively larger (340-900 aa) and adopts a major portion of the protein which could structurally be subdivided into two subunits. At the interface of these subunits a large cavity potentially for the binding with RNA is present. Sequence alignment, comparison of pockets dimensions and carbon α superimposition of NSP1 of GCV over other orthologous proteins of representative flavivirus points to the structural and functional novelties in NSP1 of GCV virus.

Key words: NSP1, GCV, Flavivirus, Molecular Modelling,

Exploring Potential of Horizontal Gene Transfer in Lux Operon System

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Horizontal Gene Transfer (HGT) is an important evolutionary force that sculpted the bacterial genomes and their existing metabolic potential. This study is designed to investigate potential of lux operon genes to be transferred horizontally in the bacterial lineage. Briefly, whole genome sequences of representative species of genus Vibrio were retrieved and GC content was measured. Then GC content of individual lux operon genes were also measured and plotted against the mean value of the genome GC content. The data show the many of the lux operon genes deviates from the ±5% window flanking the mean genome sequence, this in turn indicate the potential of lux operon genes to be transferred horizontally. To further this corresponding 16srDNA tree was developed and aligned with the cladogram of lux genes. The conflicts in the nodal arrangement also points the past events of HGT in the components of lux operon.

Key words: lux operon, HGT, bioluminescence.

Molecular Modeling and Structural Divergence of Bovine Desmosomal Glycoprotein 1

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Desmosomal Glycoprotein 1 or Desmoglein1 (DSG1) is a structural protein mainly involved in development of tissue matrix for adhesion. Recently the protein has been linked with the development of horn in *Bovidae* and/or cetaartiodactyla. Since in artiodactyla, horns exist in different forms and shape, it is potentially possible that structural differences in DSG1 may underpin phenotypic appearance of different forms of horns. To explore this at higher resolution, full length molecular models of the DSG1 of bovine was developed by iterative threading. After structural and thermodynamic refinement, the model was used as template to develop the structures of other mammalian orthologues by comparative modelling. Structural and thermodynamic assessment scores are well within reliable limit denoting the structural reliability of the model. The protein mainly comprises of multiple cadherin domains in primary structure which manifested as a repeat domains of the same in the three dimensional conformation. The domain mainly comprises multiple β sheets. Comparison with orthologous structures demonstrate noticeable deviation in the Cα backbone structure and span of the repeatitive domains which may lead to the functional divergence of the protein.

Key words: Bovidae, DSG1, Evolution, Molecular Modelling.

Partial Purification of Peptides and Proteins from Root Powder of *Glycyrrhiza Glabra* (Licorice)

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Pakistan has a number of natural resources. Medicinal plants are reported throughout the countries. Medicinal plants have increasing economic importance in the developing and developed countries. Traditional medicines and complementary medicines are known for low cost treatment of many diseases. Herbs were known the only medications for many years. Proteins and peptides use in the defence mechanism against many diseases. Extraction of many biologically active proteins, and peptides have been reported from various plants, and animals. Opiate effects, cardiovascular actions, and defensive functions are reported in peptides obtained from insects. Antihypertensive peptides have been evaluated from corn, fish, and milk protein. Immune-modulating peptides have been evaluated from rice and soybean. *Glycyrrhiza glabra* is a perennial herb, and considered as the grandfather of herbs. Previously, many phytochemicals i.e. stilbenoids, saponins, isoflavones, coumarins, and flavanoids have been reported in liquorice. Many researches were reported on the roots of liquorice. Anticancer, antipyretic, antioxidant, antidepressant, immunological, analgesic, hypolipidemic, and antimicrobial activities have been evaluated. To date, no data is reported on the purified peptides and proteins from *Glycyrrhiza glabra* roots. Therefore, the focus of this work will be on peptides and proteins extracted from liquorice roots. Proteins were extracted using 0.1% acetic acid, 25 mM sodium phosphate pH 6.5, and 20 mM Tris/HCl pH 8 buffers, and were partial purified using gel filtration chromatography. These partially purified proteins will be further purified using other chromatographic techniques. These purified proteins and peptides will be characterized by MALDI-TOF MS/MS, Q -TOF LC MS/MS, and N-terminal amino acid sequencing. Their biological activities will be evaluated by using cell culture based anticancer assay using MCF 7 Brest cancer cell line, and antimicrobial activity.

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Extraction and characterization of bromelain from Pineapple (Ananas Comosus) waste.

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Nature is a rich source of plants having tremendous medicinal properties and their products are of great importance for the treatment of various disorders and for industrial use. Among the plants that are traditionally known for having a wide array of potential bioactive compounds Ananas Comos (also called as pineapple) hold a valuable position. Pineapple is a part of Bromeliaceae family and it is consumed as food as well as folk medicine for the treatment of various diseases. It is reported that pineapple is a rich source of Bromelain which is a cysteine protease and is reported to have significant therapeutic activities like anti-cancer, anti-inflammatory, anti-thrombotic etc. it is also being used in food, pharmaceutical, cosmetics and other industries. It is present in stem, core, crown, leaves and peels which constitute the waste of the pineapple plant. Therefore, the proposed study aims at utilizing the waste of pineapple for the extraction of a vital protease i.e. bromelain. Characterization of bromelain extract will be done via different biochemical techniques and its proteolytic activity will be compared with the commercially available bromelain.

Keywords: Pineapple waste, Bromelain, proteases, SDS-PAGE.

Detoxification of Synthetic Azoic Dyes by Ligninolytic *Aspergillus niger* QMS-6 in Bioreactor Systems

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Textile effluents hold a huge amount of toxic coloured compounds having high COD concentrations and disrupting aquatic life being discharged to the environment without safety treatments. Routinely used conventional treatment methods are found to be inefficient for the secondary treatment of wastewater. Bioremediation of industrial effluent by ligninolytic immobilized fungi serves as an effective method to substitute the conventional removal processes of hazardous compounds. The present study was aimed to develop a bio treatment process using immobilized *Aspergillus niger* QMS-6 to treat the industrial effluent containing dyes in a self-designed lab scale stirred tank and trickling bed bioreactor. A ligninolytic fungal strain, *Aspergillus niger* QMS-6, capable of producing laccase, majorly was immobilized on to the pieces of various inert supports (gravels, polyethene polymers, natural loofah sponge, used steel wool and net). The reactor operation was carried out at ambient temperatures under aerobic conditions with the 1 g L-1 of glucose and 0.2 g L-1 of ammonium oxalate provided a fastest decolorization rate, where as optimal initial concentration of dye(s), pH and temperature were 100 ppm, 6.5 and 30-37°C, respectively, of simulated effluent. The reactor was run on fill with hydraulic retention time of 12 hrs. The efficacy of the treatment was monitored in terms of decolorization percentage, and reduction in values of BOD, COD, TDS and TSS. The reduced COD levels and percentage decolorization of textile dyes resulted a more ecofriendly effluent to be discharged in community. Hence fungal immobilized reactor setup represents an inexpensive, readily available and easily maintained system for in-situ remediation of industrial wastewater.

Key words: *Aspergillus niger* QMS-6*; Immobilization; stirred tank bioreactor; hydraulic retention time*

Structural Evolution of Plant Aquaporins

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Aquaporin is an important molecular component of plant physiology given its importance in maintaining osmotic pressure with in plant, which in turn plays pivotal role in transportation and translocation. Since, plants inhabit diverse niches, it is possible that physiology of aquaporin varies and the variation may have been underpinned by structural changes. In the present study, full length molecular models of aquaporins of representative plants covering all major lineages of plant were constructed using iterative threading and comparative modelling. Assessment of models points to their thermodynamic and structural plausibility. Carbon α backbone superimposition as well as dimensions of central transportation cavity reveals noticeable structural changes in the aquaporins of different plants. The finding in summation, in turn explain structural basis of functional divergence of aquaporins in plant species.

Key words: Aquaporins, Molecular Modelling, Evolution

PRODUCTION, PURIFICATION AND CHARACTERIZATION OF THERMOSTABLE BACTERIAL a–AMYLASE BY SOLID STATE FERMENTATION OF AGRO-BYPRODUCTS

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The production of extracellular α-amylase by thermotolerant Bacillus amyloliquefaciens and Bacillus licheniformis was studied under solid state fermentation (SSF). Various agro- byproducts namely Wheat flour, Barley flour, Corn flour, Gram flour, Moong husk, Arhar husk, Mustard oil cake, coconut oil cake, Banana peel, Potato peel, Sweet Potato peel, Soybean hull, Wheat bran, Rice bran, and Sugarcane baggase were examined for α- amylase production. Among all the substrates wheat flour was found to be best substrate for α-amylase production (145.56 IU/ml) in phosphate buffer as extracting medium for Bacillus amyloliquefaciens, but in case of Bacillus licheniformis, wheat bran supported maximum growth and produced maximum αamylase (154.17 IU/ml) with Triton –X as extraction medium. Process optimization was conducted using wheat flour and wheat bran in a single parameter mode showing enhanced enzyme titre. Further, the appropriate incubation period, moisture level, incubation temperature and inoculum concentration were determined. Maximum yields of 149.62 IU/ml, 144.64 IU/ml, 173.28 IU/ml, 164.48 IU/ml were achieved by employing wheat flour as substrates with Bacillus amyloliquefaciens at temperature 37°C, pH 7, moisture content 80% and incubation period 72 h whereas Bacillus licheniformis was found to produce optimum α- amylase at temperature 40°C (168.78 IU/ml), pH 6 (170.34 IU/ml), moisture content 80% (171.89 IU/ml) and incubation period 48 h (155.06 IU/ml). The phosphate concentration was also found to enhance α- amylase yield. Media supplementation with carbon source as (1%) maltose and (0.15 M) inorganic source (ammonium chloride) in SSF medium increased amylase enzyme yield (167.44 IU/ml, 167.11 IU/ml) for Bacillus amyloliquefaciens and (178.46 IU/ml and 172.36 IU/ml) for Bacillus licheniformis, respectively. The effect of addition of external organic nitrogenous compounds further showed a positive impact on enzyme synthesis by both the culture. Increase in the enzyme activity was obtained when tryptone and soy peptone at 1% concentration was added to the fermentation medium. The enzyme was partially purified to 2.40 and 2.18 fold by ammonium sulphate precipitation and ion exchange chromatography. The stability profile of the partially purified enzyme from both strains exhibited maximum activity at 65°C and pH 6-7. The enzyme exhibited marked increase in activity in presence of metal ions (Ca2+ , Fe+++, Mn2+). On the basis of comparative production optimization parameters the molecular weight of purified alpha amylase enzyme from Bacillus licheniformis was determined and estimated as 71 kDa on SDS Polyacrylamide gel electrophoresis. The apparent Km and Vmax of alpha amylase enzyme from Bacillus amyloliquefaciens for soluble starch were estimated to be 0.085 mg/ml and 212.76 IU/ml respectively whereas from Bacillus licheniformis were found to be 0.074 mg/ml and185.87 IU/ml, respectively.

Keywords: Bacillus amyloliquefaciens, Bacillus licheniformis, SSF, Enzyme activity.

Anticandidal Potential of *Bacillus* Species: Isolation, Screening and Characterization

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Candidiasis is an opportunistic fungal infection caused by yeast that belongs to genus *Candida.* There are over 20 species of *Candida* that cause infections in humans, while the most known specie that cause infections is *Candida albicans*. However, more recently other non-*albicans Candida* species are emerging as potential pathogens. Most of the antifungal drugs available for the treatment of candidiasis have been becoming useless because of the emergence of resistance against them, particularly this antifungal resistance is more higher among non-*albicans Candida* species as compared to *C.albicans*. Therefore, researchers have been working to develop some alternative agents to treat candidiasis. This study was conducted to determine the production of anticandidal metabolites by *Bacillus* species isolated from different sources such as soil. A total of ten *Bacillus* species were isolated and were investigated for bioactivity against *Candida* species. Among them, four different strains showed excellent anticandidal activity against *Candida* species which were then selected for further analysis. These *Bacillus* species were identified by conventional methods and grown in Brain Heart Infusion broth to produce cell free supernatant (CFS) after centrifugation. This CFS was tested against ten strains each of *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida rugosa* and *Candida tropicalis* by using agar well diffusion method for their bioactivity. The *Bacillus* SS04 showed maximum activity against 90% of *Candida albicans*, while *Bacillus* SS02 and SS04 showed 80% and 60% activity against *C.albicans* respectively. However, *Bacillus bodius* SS01 did not show any activity against *Candida albicans*. The metabolites produced by selected *Bacillus* species were also checked against *Candida rugosa* and it was observed that the *Bacillus bodius* SS01 showed maximum bioactivity against 60% of *Candida rugosa*. While the *Bacillus* SS03, SS02 and SS04 showed bioactivity against 50%, 30% and 20% of *C. rugosa* strains respectively. Against *Candida krusei*, it was observed that *Bacillus* SS02 showed activity against 50% of C. *krusei* strains. In the study, C*andida glabrata was also tested* and the maximum bioactivity against it was showed by *B. bodius* SS04. In case of *C. tropicalis*. The maximum bioactivity was exhibited by *B. subtilis* SS01 against 20% of isolates while the *Bacillus* SS02, SS03 and SS04 also showed activity against 10% of *C. tropicalis* strains. Furthermore it was also found in the study that the bioactivity of *B. bodius* SS04 was retained up till 60°C while lost at high temperature (80°C, 100°C, and 121°C). Moreover, this bioactivity of *B. bodius* SS04 was found only at neutral pH i.e. 7.0. The promising results obtained suggests further research in the purification and application of these metabolites in order to investigate their possible role in treatment of candidiasis in future.

Key words: Candida, Candidiasis, *Bacillus* species, Anticandidal metabolites.

Purification and characterization of low molecular mass proteins/peptides from Arugula (*Eruca Sativa*)

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A popular cruciferous vegetable named as *Eruca sativa* (Arugula), belongs to Brassicaceae family. It is usually cultivated in the Middle East and consumed both as cooked, and raw. Its biological extracts as well as essential oils consists of bioactive compounds that are responsible for nutraceutical properties. Seeds of Eruca sativa contain phytochemicals which exhibit various biological activities including anti-inflammatory activity, antimicrobial activity, and anti-cancer activity. Furthermore, these phytochemicals also provides defensive properties to plant against various pests, and pathogens.

This study deals with the purification, and characterization of low molecular weight proteins, and peptides from *Eruca sativa.* First, protein was extracted in Tris buffer followed by precipitation using ammonium sulphate. The precipitate obtained was subjected to the size exclusion chromatography (SEC) for separation of protein on basis of molecular size. Protein peaks obtained from SEC were analysed electrophoretically using SDS-PAGE. After SDS-PAGE analysis, a single fraction was further purified using RP-HPLC which gave peaks of different intensities. These fractions were digested into short peptides using enzyme trypsin which was further separated, and analysed using mass spectrometry. The data obtained was compared, and analysed using MASCOT.

Two different bioassays were including anti-bacterial (MABA), and anti-cancer (MTT) assay were done in order to determine their defensive properties. The results showed that it is active against *Staphylococcus aureus,* thus, provides protection against diseases such as pneumonia, and toxic shock syndrome. Moreover, it also showed significant anti-cancer activity against lung cancer cell line named as NCI-H460 in a dose dependent manner.

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Biofilm formation by indigenous clinical isolates of *Candida albicans*

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Biofilm formation is an important virulence factor which contributes in pathogenicity of different microorganisms. Because of this ability, different microorganisms can grow on medical devices such as dentures, vascular and urinary catheter and pose a serious threat for hospitalized patients. This study was designed to evaluate potential of biofilm formation among clinical strain of *Candida albicans* isolated from cases of Candidiasis in Karachi city. For this purpose a total of 100 isolates of *C. albicans* were obtained from Lab of Molecular Microbiology and Mycology Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi and re- identified by conventional methods such as microscopy, colonial characteristics on Corn meal agar, Germ tube test etc. The isolates were further screened for qualitative assessment of biofilm formation by using Tube staining assay. The Quantitative assessment of biofilm formation was carried out by reading its absorbance at 450 nm in spectrophotometer. The results were also analyzed statistically by using SPSS Version 16.0. The results of this study revealed that 80% of *C. albicans* isolates were positive for biofilm formation and this activity was found higher among isolates collected from urine samples of female patients. The high potential of biofilm formation among clinical isolates suggests proper management of these cases and it is required to design strategy to combat this virulence and its consequences.

Keyword: *Candida albicans,* virulence factors, Biofilm

Detection of enzymatic virulence factors in clinical isolates of *Candida albicans*

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Candida, a normal commensal of the mucous membrane and skin of human , has the ability to become an opportunistic pathogen and cause life threatening infections particularly in immuno compromised individuals.. *Candida albicans* is considered as the most pathogenic specie of genus Candida and is the leading cause of candidiasis in immunocompromised and immunocompetent individuals. The high rates of morbidity and mortality due to candidiasis are because of certain virulence traits such as adherence, dimorphism, production of hydrolytic enzymes etc. possess by Candida species. These factors facilitate Candida in invasion, deep penetration and establishment of infection in the host body. This study was focused on the assessment of certain enzymatic virulence factors such as esterase, coagulase, gelatinase and catalase production by *Candida albicans*. For this study a total of hundred clinical isolates of *Candida albicans* were obtained from Lab of Molecular Microbiology and Mycology, Department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi,, and re- identified by conventional methods such as microscopy, colonial characteristic on Corn meal agar, Germ tube test etc. These isolates were evaluated for the production of Esterase enzyme by using the Tween 80 opacity test, while coagulase production by these isolates was detected by slide coagulase technique. Similarly, the gelatinase activity of these isolates was assessed by gelatin inoculation technique in gelatin media. Similarly the catalase enzyme production was analyzed by using slide techniques. The results were also analyzed statistically by using SPSS Version 16.0. Among all the enzymatic virulence factors, catalase enzyme was predominantly produced by 85% of the Candida isolates. This rate was followed by Esterase production which was found positive in 75% of the isolates. Moreover the Gelatinase and coagulase production was also detected in 60% and 50% of the total isolates respectively. Detection of appreciable number of clinical isolates of *C.albicans* with significant ability of hydrolytic enzymes production suggest strict monitoring of these virulent strains to prevent aggravation of Candidiasis in population of Karachi city.

Keyword. *Candida albicans*, Virulence enzyme factors, Esterase, Catalase, Coagulase and Gelatinase

Aligning Buss Perry scores for Aggression with serotonin level of young individuals

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Aggression is a form of human behaviour with a spectrum of manifestations. Role of neurotransmitter such as serotonin and dopamine are linked with development and progression of aggression. However to date alignment of psychological metric with their physiological feature (serotonin level) is not conducted for aggression in a detailed and composite manner. The primary aim of the present study is to align serotonin level of least and most aggressive young individuals assessed by Buss Perry Questionnaire. Briefly, Buss Perry Questionnaire was first standardized and later distributed among 2200 young individuals between age ranges of 18-25 years. Of these, 200 filled forms were excluded due to incomplete information. The scores were than compared and stratified. Individuals with Buss Perry scores $\leq$60 were considered least aggressive, whereas, individuals with buss Perry scores $\geq$ were considered as highly aggressive. Serotonin levels was measured by ELISA of 40 individuals each from least and most aggressive individuals. Statistical analyses was conducted depending on the nature of data by Graph Pad prism. No significant difference between the scoring of Urdu and English version suggests uniformity of the questionnaire. Mean value of total score of male and female subjects was 79.14$\pm$ 16.22 and 75.28$\pm$16.97 respectively with significant difference ($p=0.0011$). Positive correlation was found between scores of different forms of aggression ($p<0.0001$). Serotonin levels of male and female individuals were also found to be significant different ($p=0.0004$) as well as between least and highly aggressive individual ($p=0.044$). Similarly, negative correlation was found between Buss Perry scores and serotonin levels ($p=0.007$) suggesting drop in serotonin level may lead to development and /or progression of aggressive behaviour. The findings are the first in Pakistan to align the levels of serotonin with psychological assessment of aggression. The study could further be extended by measuring levels of other neurotransmitters.

Keywords: Aggression, Buss Perry, Serotonin, ELISA, Behaviour.

Estimating Selection Pressure in Bacterial Lux Operon System

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Proteins encoded by lux operon work in combination to be manifested as bioluminescence in bacterial system. The trait ensue significant evolutionary advantage to the bacterial population in terms of tropisms to variety of physical and chemical factors. The present investigation deals with estimating of selection pressure of the constituent genes of the lux operon. Briefly, the orthologous sequences of the genes namely, luxA, luxB, luxC, luxD, luxE, luxG and luxR were aligned under default parameters of ClustalW. The alignment then be used to estimate the number of synonymous mutations (dS) and non-synonymous mutations (dS) and the normalized differences were deduced. Employing M0 and M1 hypotheses, inference was developed for positive and negative selection at each site. The findings show that component genes of lux operons have undergone considerable variation in the selection pressure where the structural genes are under strong negative selection with occasional signal of positive selection. In comparison, the regulatory genes were found to be mostly under neutral and/or positive selection.

Key words: lux operon, selection pressure, bioluminescence.

Estimating Selection Pressure of Genes Linked with Artiodactyla Horns

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Horns are the important structural innovation in mammalian lineage of artiodactyla. It brings several considerable evolutionary advantages in terms of defense and reproduction. Recently, several genes like *DSG1*, *DSC1* and *DHR* have been linked with the development and formation of horns. Since horns exist in the mammalian lineages in different forms and shape, it is conceivable they may have subjected to different forces of natural selection. At the finer resolution, these forces like other traits must have sculpted the genes which may consequently underpin the phenotypic variation in mammalian horns. To explore this possibility, orthologous nucleotide sequences of genes linked with the horns formation and/or development were retrieved and aligned. The alignment then used to measure the number of retained synonymous (dS) and non-synonymous mutations (dN). The normalized difference between dN and dS was estimated and then employing Mo and M1 hypothesis selection pressure of different genes were deduced. Holistically, the findings suggest strong negative selection across many genes in relation to the development of horns, however subtle regional and domain variations were observed which may in turn explicate the phenotypic differences in mammalian horns.

Key words: Horns, Evolution, Selection Pressure, Artiodactyla

Investigating C-level Paradox in Bacterial Kingdom

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C-level paradox is an interesting numerical anomaly exist in different life forms. It deviates from general understanding that more evolved the organism, the size of the genome and number of genes may be larger. However, this assumption has been deviated in several lineages and species. To date such information is lacking for the bacterial kingdom. In this study 16srDNA sequences of representative of major bacterial taxonomic groups were retrieved and phylogenetic tree was reconstructed to delve out their evolutionary relationship. A time line is drawn based on the nucleotide substitution rate in order to point the divergence time. Subsequently, the time line then be exploited to statistically analyze the progression of size of genomes and number of genes in bacterial kingdom. The data shows that C-level paradox does exist as well in the bacterial lineage especially in the two of the largest bacterial lineages namely Firmicutes and γ-Proteobacteria. This in turn represent that existence of C-level paradox is a universal evolutionary feature that encompass both prokaryotic and eukaryotic lineages.

Key words: lux operon, C-level paradox, bioluminescence.

Study of synthetic cosmetics azo dyes: A Computational approach

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Colour plays a decisive role in the marketing of a cosmetic product in which azo dyes are one of the most important classes which are widely used in different industries and also owing to their stability and ease of synthesis. Azo dyes are commonly ingested and come into contact with skin by numerous sources like food, cosmetics and drugs. These dyes are reduced by many aerobic and anaerobic bacteria from normal skin flora of human. Azo1 azoreductase gene from Staphylococcus aureus is a FMN-dependent enzyme responsible for the degradation of azo dyes. In this study, Homolgy model for azoreductase enzyme from S.aureus was generated and validated. Molecular docking was performed with the azo dyes for binding energy calculation and active site residues were identified. The ionization constant (pKa) of the azo dyes were determined and structure optimization of synthetic azo dyes were based on the quantum chemical calculations performed by Density Functional Theory (DFT). The in silico results were in good agreement with experimental studies. It is hoped that this study will provide essential understanding for the design of new and harmless azo dyes.

Key words: Azo dyes, Azoreductase, Molecular docking, pka , DFT

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HPLC based decomplexation of Cobra (*Naja naja naja*) snake venom

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Snake venom is a complex mixture of proteins and peptides responsible for the life threatening bite to the victim. Various protein components and other toxins are isolated and characterized from different species of snakes in Pakistan. This study is based on the exploration of variation in cobra snake venom toxin due to diverse geographical distribution in the country. Here we present the HPLC separation pattern of Cobra snake venom specimen collected from province of Sindh. Crude venom was analyzed on SDS PAGE and applied to HPLC attached with reversed phased C18 column. The fractions were collected and again analyzed on SDS PAGE to observe the fractionation pattern of proteins and peptides in the venom.

Transfer of *bla*$_{CTX-M-15}$ and *qnrS* genes from MDR *Salmonella enterica* serovar *typhi* to XDR strains of Salmonella through a plasmid lncY among the population of Karachi

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Background: Typhoid fever caused by *Salmonella typhi*, remains a significant public health problem in developing countries. Antibiotic-resistant *S.typhi* strains have become increasingly common. The first XDR of *S.typhi* reported in Hyderabad then transmitted to Karachi, which are difficult to treat by many of the antibiotics. Each week there were a number of Typhoid fever reported in the different hospitals of Karachi city.

Objective: The aim of the study is to the detect the *bla*$_{CTX-M-15}$ and *qnrS* genes in the XDR strains of the *S.typhi* mediated by Plasmid, responsible for the resistance of organism to Third-generation Cephalosporin and Flouroquinilones which are not found in MDR strains.

Methods: 71 *S.typhi* strains collected from different hospitals of Karachi from July-2019 to October-2019, Demographic features were recorded, the identification is confirmed by Biochemical testing. Kirby Bauer disc diffusion method was performed to check the susceptibility testing, focused against third generation-Cephalosporin and Flouroquinilones. Extracted DNA (Colony boiling) were checked by PCR technique for the presence of *bla*$_{CTX-M-15}$ and *qnrS* genes. Plasmid *lncY* was extracted by using kit (ThermoFisher) from XDR strains of *S.typhi*.

Results: On the basis of collected strains, results proved that males (59%) are more prone to the typhoid then females (38%), 87.23% cases were found to be in the age group of 1 to 15 years . 80% of the collected strains were resistant to Third-generation of Cephalosporin and 68.6% strains were resistant to Flouroquinilones considered as XDR which were confirmed by the presence of bla$_{CTX-M-15}$ and qnrS genes in PCR reactions. All the strains (100%) were Sensitive to Meropenum 98% strains were resistance to Azithromycin. Only about 20% strains were proved to be MDR. Extracted plasmid lncY was not found in the MDR strains, proved that the resistance in XDR is plasmid mediated.

Conclusion: The Children ages upto 1 to 15 years are at high risk for typhoid fever and more prevalent in the Males in Karachi. The *bla*$_{CTX-M-15}$ and *qnrS* genes responsible for the resistance of *S.typhi*, mediated by Plasmid lncY which may be arises from the environmental origin through conjugation. Further research is required to find out the actual origin of generating XDR in many of the bacteria.

Keywords: *Salmonella typhi, bla*$_{CTX-M-15}$ gene, *qnrS* gene, Plasmid *lncY*, Third generation cephalosporin.

Proteomic Analysis of Oxidatively Modified Proteins in Schizophrenia

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Schizophrenia is a complex disorder caused by multiple factors, in which oxidative stress is found to be an important consideration. Several pro and anti-apoptotic proteins and factors are found to be involved in oxidative dysregulation. Dysregulation of oxidative stress can lead to disturbed cell metabolism and finally to apoptosis via mitochondrial pathway. Oxidative stress may leads to protein modification including 4-hydroxy nonenal (4 -HNE), carbonylation and 3-nitrotyrosine (3-NT), which may contribute in the progression of schizophrenia. Present study identifiy those modified proteins and their respective metabolic pathways which are contributing towards pathophysiology of schizophrenia. For this purpose, seven autopsied schizophrenic brain samples were subjected to western blotting for the detection of HNE-modified proteins. Additionally,Thiobarbituric acid substances (TBARS), reduced glutathione (GSH) and catalase (CAT) activity were also estimated in brain samples. Increased TBARS level and expression of HNE modified proteins in schizophrenic brains shows increased lipid peroxidation LPO and can be used as an index of oxidative stress as it reacts with malondialdehyde MDA which may conclude that antioxidant defense system was found to be altered due to an increased oxidative stress. Elucidation of HNE modified proteins and their functional role in cellular and molecular pathway will help to explore novel drug targets and better therapeutic strategies for schizophrenia.

Synthesis of Silver Nanoparticles (AgNPs) and their Effects on blood Hematological parameters: study in rats

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Background:
The aim of this study was the synthesis of silver nanoparticles by most common chemical reduction method and to investigate the effects of its doses on blood parameters in Albino Wistar female rats.
Method:
A chemical reduction method was used for the preparation of silver nanoparticles by using $AgNO_3$ solution, Citric acid, Hydrazine Hydrate and Sodium Dodecyl sulfate (SDS). The product was characterized by Scanning Electron Microscopy.
Twelve female rats were investigated to observe the effects of chemically synthesized silver nanoparticles. The test group was treated intraperitoneal (IP) at doses of 500 mg/kg body weight diluted nanoparticles in distilled water twice in a week and blood samples were collected in EDTA coated tube to check hematological effects.
Result:
The image by SEM has shown needle like shaped nanoparticles, size 80.3 to 156nm
Significant dose related changes in hematology of female rats were noticed. Decrease in WBCs, granulocytes, lymphocytes, monocytes, MCHC, PDW, P-LC,MPV and RDW were observed and increase in hemoglobin, RBCs, MCV, MCH,HCT,PLT and PCT were noticed.
Conclusion:
Chemical reduction method was used for the synthesis of silver nanoparticles and the effects of the synthesized nanoparticles were reported, systematic toxicity in blood parameters, thrombocytosis and polycythemia were observed.

Key words: $AgNO_3$, EDTA, Hematology, Thrombocytosis, Polycythemia, Chemical reduction

“PRIMeasy” an android solution for primer designing

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Primer designing is a key concept in amplification of DNA segment through polymerase chain reaction (PCR). A number of web application are available for primer designing but an android application for this purpose has not been introduced until now. We have developed the android application name “PRIMeasy” which designs appropriate primers from provided DNA sequence considering the primer length, GC content, 3’ stability and other quality parameters. To the best of our knowledge, “PRIMeasy” is the first android application design for this purpose and would be helpful for biologist working with PCR amplification.