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ABSTRACT BOOK

Biochemistry for better food, health and environment in the developing world

Organized by:

Department of Biochemistry
University of Agriculture Faisalabad-Pakistan

PLENARY LECTURES

Plenary 1: Designing Proteins by Addition, Deletion, Substitution and Fusion

M. Waheed Akhtar

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Advances in recombinant DNA technology combined with the large variety of protein engineering methods, *in silico* tools and high-throughput screening techniques allow unlimited possibilities of improving proteins and enzymes for applications. Several methods including site-directed mutagenesis, error prone PCR, *in vitro* recombination, usage of specific substrate-binding modules, truncation of molecules, and fusion of selected protein segments has been used to modify the expressed recombinant proteins. Glycosyl hydrolases commonly contain the carbohydrate binding modules (CBMs), which may be quite specific for activity. For example, in the case of the endoglucanase CelA of *Clostridium thermocellum* the binding module CBM3a when attached to the n-terminal of the catalytic domain (CD) is more than twice as active as the variant having the binding module attached to the C-terminal of the CD. In the case of xylanase XynZ of *C. thermocellum* deletion of CBM6, which is found in association with the CD in the native state, enhances the activity manifold. Truncation of the endoglucanase CelX.C from an extreme thermophile, by removing 11 residues from the C-terminal, resulted in 5-fold increase in activity and the product expression in *E. coli* was transformed from insoluble to a soluble form. Substitution of amino acid residues through error prone PCR of the gene encoding the enzyme can result in several-fold increased activity as we have shown in the case of an endoglucanase of a *Clostridium* species. Fusion of fragments from two or more proteins can produce molecule showing the specific characteristics of the donor molecules. For example we have developed a series of fusion molecules with epitopes from two or more antigens of *Mycobacterium tuberculosis* for use in serodiagnosis of tuberculosis. The nature of the activity center residues and their positioning in the newly produced molecules are important for achieving high activity or binding to other proteins.

Plenary 2: The technology development of storage and processing for fruit, vegetable, root and tuber crops in Sichuan, China

Xie Jiang

Institute of Agro-products Processing Science and Technology of Sichuan Academy of Agricultural Sciences (SAAS), China

Based on the different climate condition from high mountain to low land of hot-wet and cool-dry type in Sichuan Province, China, the new technologies and traditional storage facilities have been improved by utilizing the advantageous outside temperature and humidity for fruit, vegetables, root and tuber crops in small and medium scale, by means of semi-underground house, cave and the house on ground with the fair well controlled RH and micro organism. Then, followed by the integrated cooling and CA storage house with the auto control of temperature, RH, O_2, CO_2 and ozone as a complete engineering and technical system for large scale storage of orange, kiwifruits, apple, lemon, cherry, pear, peach, plum, pomegranate, vegetable, potato and sweetpotato. The storage guarantees the prolonged supply for the fresh consumption, seeds and processing up to 6-8 month. One of the root and tuber crops' processing achievements by SAAS are successful industry system from refined sweetpotato (SP) starch, whole instant noodle, extruded and baked snack foods to flour and its derived products. By energy saving technology of flash drying, the SP flour and the puree are made, and further baking and double screw extruding technology have been newly researched and used for the series of derived foods such as instant flour, baked food, noodle and cookie. In instant food R&D, the new complex sterilization of 2450Hz microwave treatment have made it possible to maintain the good flavor of food quality as well as longer shelf life, and the hygienic work shop and processing procedure control technology have become one of the most important factors for the production of baked foods, the famous "Sichuan pickle "of lactic acid bacteria fomentation product and many other fruit and vegetable foods.

Plenary 3: Drug-loaded stem cells- a novel cancer/brain therapeutic?

Mark Slevin

School of Healthcare Science, Manchester Metropolitan University, UK

Recent evidence has demonstrated that stem cells are able to take up and perofm timed-release of a variety of drugs making them potential candidates for drug delivery. Here I will present data discussing the mechanisms of delivery and possible targets including cancer therapy and recovery from acute stroke. Primary data will be included showing characterization of mesenchymal stem cells loaded with a variety of drugs and combinational therapies discussed.

Plenary 4: BIOREFINERIES IN ASIA – new developments in challenging times

Wolfgang Baltus

PRECISE Corporation, Bangkok, Thailand

The presentation will give an overview about the status of Biorefineries in global context with focus on the development in Asia. The current market for bio-based chemicals is still small but expected to grow with a CAGR of about 7% until 2020. In 2013 the total production capacities of bio-based polymers was about 5 million tons – this means 2% of the total polymer production capacities. The economic development with low oil prices and the shale gas boom, however, have a strong impact on the development and marketing of bio-based materials. Biorefineries and so called "Biohubs" are seen as a competitive way to develop these industries by combining biofuels, biochemical and biomaterials in a sustainable approach. Current concepts and policies, which include the integration of bio-based chemicals and the utilization of $2^{nd}/3^{rd}$ generation feedstock following the current global development trend, will be discussed on the example of selected Asian countries including Thailand, where Biorefineries are established for many years. New approaches like the establishment of bio-gas biorefineries will be highlighted as these kind of smart integrated factories are expected to contribute significantly to the society by the introduction of smart community concepts in combining bio-based energy with bio-based material supply. The presentation will include an overview about new technology developments from agriculture feedstock to "waste" materials like carbon dioxide, methane or organic foodwaste and their potential to produce biochemical and bio-based polymers.

Plenary 5: Defining Ethical Boundaries of Health Biochemistry and Biotechnology: Restriction Endonucleases to Restricting Humans through Gene Editing

Muhammad Mukhtar

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Elucidation of biochemical processes were crucial in the development of several therapeutic agents, like a clear understanding of cholesterol metabolism that has helped in the development of statins the cholesterol depleting drugs saving lives of millions. Similarly, highly purified restriction endonucleases utilizing sophisticated biochemical techniques led to deciphering biocode of human life. Our laboratories are involved in understanding biochemical and molecular aspects of neurological disorders afflicted by infectious agents utilizing cutting edge procedures. Here we present data emanating from comparing two set of research avenues pursued in our laboratories i.e. our team research efforts in understanding the role of cholesterol in the passage of infectious agents into the brain ensued highly beneficial human clinical trials at the concept of new use of old inventions (Phase IV clinical trials), whereas, efforts by us and several other laboratories of the world in targeted gene delivery emerged in the form of powerful gene editing technology. The gene editing biological system is comprised of two components, a guided RNA. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and an endonuclease, CRISPR associated (Cas) nuclease called Cas 9. Utilization of the CRISPR/Cas9 system resulting in editing of human genome led to emergence of ongoing ethical debates all across the world. Comparing both domains of research i.e. cholesterol metabolism and targeted gene delivery, and knowledge generated from research in allied disciplines, we identified several ethical dilemmas that need to be resolved particularly in the developing and underdeveloped world taking into consideration, social, cultural and religious values. For example mitochondrial replacement therapy from a surrogate woman leading to baby having genomic material from two women and one man approved in United Kingdom [Power play- *The replacement of mitochondria does not signal ethical problems.* Nature, 2015 Sep 24; 525(7570):425-6] will be acceptable in various social and religious values. Based on solid body of evidences and our previous visionary thoughts in ethical domains of scientific research, we propose a global ethical education program defining direction for ongoing and new research avenues before humane humanity values start getting distorted.

Plenary 6: Small Natural Molecules Based Drug Discovery and their Biotechnological Production

Athar Ata

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The University of Winnipeg, Canada

Plant natural product chemistry has played an active role in generating a significant number of drug candidate compounds for the drug discovery program. For instance, taxol, one of the best known anticancer agents was isolated from a Pacific yew tree, *Taxus brevifolia.* Enzyme inhibitors are important in discovering pharmaceuticals against Alzheimer's disease and cancer. Acetylcholine serves as a neurotransmitter in the central and pheripheral nervous system. Acetylcholinesterase (AChE) stops the function of acetylcholine by its hydrolytic destruction. Alzheimer's disease is a progressive degenerative neurologic disorder that results from the deficit of cholinergic function in brain. Enhancement of acetylcholine level in the brain is considered one of the most promising approaches for treating Alzheimer's disease. The use of potent inhibitors to inhibit AChE activity is one of the methods to enhance acetylcholine level in brain to cure Alzheimer's disease. Glutathione *S*-Transferases (GST) are phase II detoxification isozymes that function in the conjugation of a wide variety of exogenous and endogenous electrophilic substances to glutathione. Glutathione is a tripeptide having ɣ-glutamyl-cysteinyl-glycine amino acids in its back bone. This glutathione adduct formed is a less toxic and have very high solubility in water. This adduct can easily be excreted from the body. Anticancer drugs have electrophilic centers and can easily form this adduct in the presence of GST and will be excreted from the body. This would lower the efficiency of chemotherapeutic agent. To increase the effectiveness of cancer chemotherapeutic agent, it might be necessary to use GST inhibitors as adjuvant during chemotherapy. Our recent phytochemical studies on medicinally important plants have resulted in the isolation of natural products exhibiting health related enzyme inhibitory activities. In this talk, we will discuss the structure elucidation of these new natural products and their bioactivity data. Structure-activity relationships of these compounds and our approach to develop new biotechnological methods to produce novel bioactive natural products will also be discussed.

Plenary 7: Streamlining clinical trial regulations and infrastructure are attracting the clinical trial industry in the UK- A lesson for Pakistan

Tanweer Ahmed

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In the UK, there have been significant changes in clinical research regulations over the last few years. In order to run multi-centre clinical trials, researchers were required to apply from a number of Local Research Ethics Committees (LREC). Though gaining approval from local research ethics committee was satisfactory to some extent, the researchers and industry were not happy due to the time it took and in particularly, the inconsistency in gaining approval from different LRECs. Due to inconsistency and delays in gaining approval, in 1997, Multi-site Research Ethics Committees (MREC) were established. Once approval was given by the MREC, the researchers were still required to apply from LREC. There was some improvement but it was still found to be time consuming, costly and bureaucratic. Due to complaints by researchers and industry, central Offices for Research Ethics Committees (COREC) was established in 2000. In 2009, the Integrated Research Application System was established to further streamline approval process. In March 2010, the UK Government asked the Academy of Medical Sciences to undertake an independent review of the regulation and Governance of health research. As a result of their findings, Health Research Authority (HRA) was established. At present, HRA is introducing a number of changes to make the UK an attractive place for clinical research. Due to streamlining of regulations and clinical trials infrastructure provided by the National Institute for Health research (NIHR), there has been a 40% increase in commercial studies since 2010. In addition, there has been significant improvement in approving studies in a timely manner and ensuring studies are delivering patient recruitment targets. Pakistan can significantly benefit from learning and implementing similar changes.

Plenary 8: Agri-based Bioeconomy

Khurshid Saharan

Senior Markets and Trade Officer, Agriculture and Agri-Food Canada (AAFC), Canada

The talk will cover a brief introduction to the North American bioeconomy. The talk will be focusing on Canadian agri-based Bioeconomy and its Stakeholders including private sector and the Federal and Provincial Governments. A summary on the major R&D Consortia including a synopsis on the Regional Clusters across Canada dealing with bioeconomy will also be deliberated. The overall Canadian energy production and consumption trends will be discussed including renewable energy production and feedstock use for energy and non-energy applications – Both Federal and Provincial Renewable Energy Policies including the biofuel mandates will be discussed. The total supply of biomass feedstock, including forestry, and agriculture crops and the crop residues. An overview will be provided on the supply chain of fuels and innovative bio products, highlighting Canadian facilities including running commercial biorefineries, biorefinery demonstration and pilot plants. The discussion will include an overview on the major national stakeholders involved in the field of biorefining biofuels and biogas – the markets, products co-products.

TRACK 1

CLINICO-MEDICAL

AND

PHARMACEUTICAL BIOCHEMISTRY

TRACK COMMITTEE

1. **PROF. DR. KHALIL-UR-RAHMAN (FOCAL PERSON)**
2. **DR. FATMA HUSSAIN**
3. **Dr. KASHIF JILANI**

KEYNOTE LECTURES

TRACK 1

K-1.1 Paradigms of the centrosomal proteins in embryonic development

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Centrosomes are the major microtubule-organizing centers of animal cells. Regulated centrosome assembly is essential for accurate cell division, organ/tissue homeostasis and for maintaining genome integrity. Centrosomal genes are targeted by mutations in numerous human developmental disorders like primary microcephaly, where only the brain size is severely reduced. We used the combination of homozygosity mapping and whole-exome sequencing to identify the novel causative genes in a pool of primary microcephaly and primordial dwarfism. Post-hoc capillary sequencing of each respective variation corroborated the identification of mutations as the causal variants. Autosomal recessive primary microcephaly (MCPH) is a neuro developmental disorder resulting in diminution of brain growth in utero. Obvious feature of the patient is reduced head circumference (HC) associated with mild to moderate intellectual disability. MCPH is heterogeneous with 13 known loci: MCPH1-MCPH13. The underlying genes have been identified for all 13 loci which code for centrosomal and chromatin regulator proteins. We have ascertained 94 MCPH families from various regions of Pakistan. Homozygosity mapping complemented with exome or targeted gene sequencing identified several novel and known mutations in reported MCPH associated genes. Linkage were established for 63 families to known MCPH loci including 49 to MCPH5 (ASPM), 5 to MCPH1 (MCPH1), 3 to MCPH2 (WDR62),2to the MCPH3 (CDK5RAP2), 1 to MCPH4 (CASC5), 2 to MCPH6 (CENPJ) and 1 to MCPH7 (STIL) locus, whereas 31 families were unlinked. Novel and known mutations were identified in ASPM, MCPH1, WDR62, CDK5RAP2, CASC5,CENPJ and STIL. Out of 31 unlinked families, so far we identified CEP135 and CDK6 as the novel causative genes of primary microcephaly in two independent families. Immunostaining of CEP135 showed strong signals in the developing neuroepithelium of the cerebral cortex during embryonic stages E11.5 through E15.5. Fibroblasts obtained from one of the patients showed multiple and fragmented centrosomes, disorganized microtubules, and reduced growth rate. Similar effects were reported after knockdown of CEP135 through RNA interference; we could provoke them also by ectopic over expression of the mutant protein. Furthermore, we have also investigated the defects in ATR-dependent checkpoint signaling in patient cells. In a second family of primary microcephaly, we identified a mutation (c.589G>A; p.Ala197Thr) in CDK6 as the novel causative gene of MCPH12 locus. We show for the first time that CDK6 associates with the centrosome during mitosis, whereas this does not occur in patient fibroblasts. Moreover, the mutant cells exhibited supernumerary centrosomes, disorganized microtubules and mitotic spindles, an increased centrosome nucleus distance, reduced cell proliferation and impaired cell motility and polarity. Upon ectopic expression of the mutant protein and knockdown of CDK6 through shRNA we noted similar effects. Our findings suggest new genes responsible for primary microcephaly strengthen the role of centrosomes in the cause of these diseases, and place these genes among the essential components of this important organelle in particular for a normal embryogenesis.

K-1.2 *Chenopodium quinoa*: A High Nutritional and Medicinal Value Climate Proof New Grain Crop in Pakistan

Shahzad M.A. Basra and Irfan Afzal

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Pakistan is facing serious food security challenges mainly because of burgeoning population, increasing agricultural input cost and highly variable climatic conditions. Use of low-quality brackish, municipal and industrial waste water is further increasing the already very high proportion of problem soils in the country. Majority of the population has to depend on wheat for most of the calories and protein resulting in malnutrition of low income population. Quinoa was intruded as climate proof new crop having high nutritional and medicinal profile, low input and resistant to biotic and abiotic stresses. Quinoa (*Chenopedium quinoa* willd.) belongs to Andes Mountains and has been cultivated since ages. Quinoa grain has better nutritional profile than other staple foods i.e. protein (13-21%) having all essential amino acids, minerals (Fe, Cu, Ca, Zn, Mg), vitamins (B2, A, E), and good quality fat. Quinoa has heart-healthy fats like monounsaturated fat (oleic acid) and small amounts of the omega-3 fatty acid. It also acts as a natural appetite suppressant because it is effective in controlling appetite and helps to consume less food throughout the day. Therefore it is recommended for dieters due to its high protein and low calorie nutritional profile. Moreover, it is a gluten free diet. Crop is tolerant to adverse conditions like salinity, drought, frost and diseases and insect pest. Quinoa can be grown on problem soils, with less inputs, require less crop care and produce grain of very high quality with international demand. Germplasm has been screened for local conditions, already achieved almost double the yields than the world's average and comparable nutritional profile.

K-1.3 Molecular tools for detection of animal pathogens

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While traditional testing methods are still widely used in veterinary diagnostic laboratories exciting new technologies, such as biosensors and microarray techniques, are being developed. Nucleic acid diagnostic techniques such as polymerase chain reaction (PCR) have become routine diagnostic tools in veterinary laboratories not only to make specific typing determinations, but also to rapidly screen large numbers of samples during disease outbreaks. In addition, nanotechnologies, although not yet implemented in veterinary laboratories, hold the promise of screening for numerous pathogens in a single assay. Other biotechnologies are likely to be widely used in the future as they can improve diagnostic capabilities while reducing the time and perhaps, the costs, associated with conventional technologies. This lecture describes some of these new technologies and concludes that although a lot of developmental work is still required, biotechnology and its applications hold great promise for improving the speed and accuracy of diagnostics for veterinary pathogens.

K-1.4 Biometrics: A Human identification Tool

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With the increase in crime rate in society, the challenge to identify of the suspects is also increased. Forensic science provides the platform to curb the crime by finding the perpetrator. In ancient time, systematic approach to use body measurements for identification of individuals was used for the investigation of crime. With the passage of time, biometrics are developed as a reliable instrument which helps law enforcing agencies to find the individuals based upon the personal features and characteristics. Such characteristics may prove as mute witnesses against the person, who own them. These characteristics include fingers and fingerprint, ear, facial features, foot, iris, lip prints, voice, gait, dental records, handwriting, hand geometry and DNA. Biometrics are mathematical and statistical methods to analyze the biological data to authenticate the individuals. Biometric traits must meet the conditions of distinctiveness, permanence, universality and collectability. It is now developed as independent and authentic tools to assure the identity of any person. In recent years, the use of computers results in advancement in the field of biometrics. The surveillance technology also played an important role in the progress and utility of this field. Many databases are developed based on such human traits. The benefit of such databases is that the information present in these can prove useful to solve the crimes. Crimes and suspects can be linked with each other by the use of such databases.

K-1.5 management of hepatitis C patients: past, present and future

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Hepatitis C has become a real threat all over the world. About 20 million individuals are infected with Hepatitis C virus (HCV) in the world with a prevalence of approximately 6 million only in Pakistan. Proper management of Hepatitis C regarding diagnosis and its treatment always remain a great challenge especially in underdeveloped countries. From the day of Hepatitis C virus discovery till to date new inventions have been introduced to upgrade the diagnosis and treatment approaches. Different types of direct and indirectly acting antiviral medicines to treat Hepatitis C patients have been introduced on different stages in last three decades. In past, Hepatitis C patients were treated with conventional or Peg- Interferons, which have low response rate and were associated with a long list of side-effects. Although with the introduction of Ribavirin that was given in combination with Interferons response rate improved, yet most of the patients remained non responders. The recently approved direct acting antiviral (DAA) drug Sofosbuvir (SOF), opens up potent treatment regimes for chronic HCV patients. SOF is an oral nucleotide analogue inhibitor of the HCV-specific NS5B polymerase with high antiviral efficacy and a favorable safety profile. SOF is more affective for HCV genotype 3 and luckily 60-80% Hepatitis C patients in Pakistan are infected with this type. So, it's right time to get rid of Hepatitis C in Pakistan.

ORAL PRESENTATIONS

TRACK 1

1.1 Diagnosis of Hepatitis C in Pregnant Mothers and its Transfer Pattern in Neonates

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The project was designed to determine the percentage of transmission and prevalence of Hepatitis C virus from mother to neonates. Assessment of the viraemic status from the early postpartum period onwards of children born to HCV-infected mothers. Also for quantitative analysis of RNA levels in mother blood. Hepatitis is the most common liver diseases in the Pakistan caused by blood-borne infection of HCV. For the diagnosis of hepatitis C in mothers, blood samples of fifty HCV pregnant women between 23-41 years old were taken. The blood samples were centrifuged at 8,000 rpm and serum was separated and stored at 4^0C. The values of the Alanine Aminotransferase were determined at 2nd and 3rd trimester of pregnancy. After extraction HCV-RNA were transcribed and amplified by PCR. The samples were further authenticated through the Agarose Gel Electrophoresis system and bands were obtained. Nested reverse transcription PCR (RT-PCR) was conducted for the quantitative analysis of HCV-RNA. The results showed that in 66% cases, the mothers had high level of ALT at 2nd trimester of pregnancy. Their ALT level was decreased in the 3rd trimester of pregnancy. PCR results showed that 40% pregnant women had quantity of HCV-RNA in the range of 1000-10,000 IU/mL and in 18% women were above 100000. The results of spectrophotometer showed that 80% infants had the antibodies against HCV-RNA while only 20% of the neonates did not have antibody right after birth. The 29% babies got HCV-RNA in their serum and became positive for HCV-RNA.20% transmission occurs during labor and at time of delivery when blood of both mother and neonate is in contact with each other. Vertical transmission rate is lowered when mother is HCV RNA-negetive. The detection of HCV RNA in the serum of newborn babies after the three months of delivery showed that infection occurred during pregnancy.

1.2 Anti-Hyperglycemic Evaluation of N-Cyanomethyl-1, 2 Benzothiazine and its Derivatives

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Diabetes mellitus is promptly intensifying worldwide disease and number of patients is predictable to levitate 50% over the next 25 years influencing about 600 million adults. The aim is to investigate the newly synthesized compounds for the two biological activities, to recognize and achieve specific anti-hyperglycemic and anti-bacterial goals that can substantially diminish the morbidity making the effective cure of hyperglycemia and bacterial infections. N-cyanomethyl-1,2-benzothiazine and its derivatives have earned too much consideration in biological research as they possess anti-inflammatory, antimicrobial, anticancer, antitumor and anti-diuretic properties. In present study, six synthesized compounds of benzothiazine were reported to study the influence on diabetic and bacterial diseases. To evaluate the antihyperglycemic potential of N-cyanomethyl-1,2-benzothiazine and its derivatives , alloxan monohydrate; a toxic glucose analogue, was injected that selectively demolish the pancreatic β-cells and causes diabetes in experimental albino white rats. The 6 group of rats were assessed at two dose levels of 1 mg/kg and 10 mg/kg of tested compounds as drugs. Both the treated groups exhibited significant hypoglycemia except AD111-Mn when compared to 7^{th} group given standard drug; metformin at regular intervals of 15, 30 and 60 minutes which is evident from Plasma Glucose levels. To further potentiate the role of this compound, antimicrobial activity was also measured using standard disc diffusion assay against four bacterial strains in vitro to establish structure-activity relationship. All compounds especially AD111-Cu demonstrated promising antibacterial activity; though, other compounds revealed moderate to poor antimicrobial activity pertaining to the reference drug Ciprofloxacin against the test strains.

1.3 Evaluation of Metabolic Changes in Asphyxiated Infants

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Asphyxia is considered an important cause of morbidity and mortality in neonates. This condition can affect many vital organs including the central nervous system and may eventually lead to death or developmental disorders. The present study was conducted to evaluate the metabolic changes due to birth asphyxia. We had collected the data of 100 neonates (50 healthy infants and 50 asphyxiated neonates) with age range less than 1 month from children ward of Nishtar Hospital Multan, Pakistan. Data analyzed by Paired sample t-test by SPSS software. The result showed that in asphxiated neonates lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, bilirubin , urea levels were increased than healthy infants, while the platelets count was smaller in asphyxiated neonates than healthy infants. This study reveals that the due to anaerobic condition normal levels of blood analytes become imbalance which may cause dysfunctioning of vital organs of asphyxiated infant. It is concluded that measures should be taken to avoid birth asphyxia with great importance to avoid complication during labor by skilled attendance.

1.4 Incidence of Thrombocytopenia in Seropositive Dengue Patients

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Dengue has become a major health concern globally in recent decades. Dengue infected patients manifest a spectrum of symptoms and in severe cases the fate is mortality. A hallmark of dengue infection is thrombocytopenia which causes concern for the patients and treating doctors. This study aimed to evaluate the incidence of thrombocytopenia in seropositive dengue patients. Bleeding manifestation was also investigated in dengue patients to evaluate its association with the severity of thrombocytopenia. In this study, 750 individuals were screened for dengue infection by detecting immunoglobulin M (IgM) against dengue virus in their serum. Enzyme-linked immunosorbent assay (ELISA) was performed for detection of IgM antibody and 250 individuals were found to be seropositive. Platelet counts were performed on whole blood of seropositive patients using Sysmex XE-5000 Automated Hematology Analyzer. Among 250 dengue patients, 2% had severe thrombocytopenia, 65.2%were found to have mild to moderate thrombocytopenia and 32.8% had normal platelet counts. Bleeding was related to the severity of thrombocytopenia as 80% of patients having platelet count lower than25000/µl showed bleeding manifestations.

1.5 *In Vitro* Bioactivities and Sub-Acute Toxicity Profiling of Three Medicinal Plants of Family Lamiaceae

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To screen out methanolic extracts of *Ocimum. basilicum, Thymus vulgaris* and *Rosemarinus officinalis* for their toxic effects at 1000 and 1500mg/kg body weight in male albino rats**.** Standard Folin ciocalteu reagent assay and aluminium chloride colourimetric assay were employed to determine total phenolic and flavonoid contents. Antioxidant activity was measured through DPPH assay. Synergistic effects of plants extracts with two antibiotics amoxicillin and ciprofloxacin were determined through broth micro dilution method against E. coli, S. aureus and S. mutans. For toxicity assay 56 male albino rats of Sprague Dawley strain were randomly divided into seven groups each of eight rats. Rats in group A were administered orally with 1 ml of distill water. Animals in group B, C, and D were administered with 1000mg/kg body weight and in group E, F and G were given 1500mg/kg body weight of plants extracts. Weight of the rats, organs, and hematological parameters were measured after administration of plants extracts for one month. *O. basillicum* extract showed highest total phenols, flavonoids, and antioxidant and antibacterial activities**.** *T. vulgaris* extract caused hypertrophy of liver while R.*officinalis* caused atrophy of spleen at both doses. *T. vulgaris* and *O. basilicum* extracts significantly increased RBCs, packed cell volume, hemoglobin and mean corpuscular volume at 1500mg/kg body weight. *O. basilicum, T.vulgaris, and R.officinalis*are rich sources of phenolics and flavonoids having a good antioxidant and antibacterial potential and are safe for treatment of various ailments.

1.6 Analysis of Neonatal Jaundice and Assessment of Interventional Outcomes of Phototherapy in Patients of Neonatal Intensive Care Unit, PIMS, Islamabad

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Jaundice is one of the most common problem, occurs with neonates when the liver cannot clear a sufficient amount of bilirubin from the plasma. Objective of this study was to study Serum Bilirubin Levels in Neonatal Patients. This study was conducted in chemical pathology laboratory at children hospital Pakistan institute of medical sciences Islamabad .A total of 120 neonatal jaundice patients were taken admitted in the neonatal intensive care unit. Serum bilirubin was checked and any value >15mg/dl was considered as jaundice and data was analyzed using SPSS version 16. A total of 120 samples of neonatal jaundice patients were run in this study with a max bilirubin level of 39.54 and minimum bilirubin level 8.70 in which the male to female ratio was 1.5:1. The patients having the total bilirubin level >15mg/dl were 66% and the remaining 354% were those having total serum bilirubin <15mg/dl. The highest percentage (34.9%) of neonates, were those having the total bilirubin level between 16 – 20mg/dl. The study suggests that there is an inverse relation between the bilirubin and the phototherapy; it greatly reduces the level of bilirubin. All the 120 patients were given phototherapy, 114 recovered and only 6 expired out. Only 19 patients were given exchange blood transfusion. A high level of bilirubin was found in the preterm babies. Majority of the babies was those having serum bilirubin level >15mg/dl with the predominance of male and preterm babies, and phototherapy was effective in the treatment of neonatal jaundice.

1.7 Effects of *Carissa Opaca* Fruit Extract on Arsenic Induced Testicular Damage in Rats

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In the current study protective effect of *Carissa opaca* fruit extract was investigated against arsenic stimulated reproductive ailments in male Sprague-Dawely (SD) rats. Twenty rats were divided into four groups. The first group served as a control. The second group of rats was treated with sodium arsenite at the dose of 40 ppm in drinking water. The third group served as a positive control and received an oral dose of *Carissa opaca* fruit extract (200 mg/kg). In the fourth group, *Carissa opaca* fruit extract (200 mg/kg) was co-administered orally with arsenic (40 ppm in drinking water). All the treatments were carried out for 30 days. Arsenic treatment resulted in DNA fragmentation in testis of rats. In contrast, these adverse effects of arsenic were eliminated by co-administration of *Carissa opaca* fruit extract. Additionally arsenic treatment significantly increased testicular thiobarbituric acid reactive substance (TBARS) levels while catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GSR) activities, and plasma and intra-testicular testosterone concentrations, were decreased significantly. Lipid peroxidation (LPO) was significantly suppressed and depleted antioxidant defense mechanism was restored by the *Carissa opaca* fruit extract treatment. Also *Carissa opaca* fruit extract treatment resulted in a marked increase in plasma and testicular testosterone concentrations. On the basis of these findings, it was concluded that *Carissa opaca* fruit extract may be used toameliorate arsenic induced reproductive toxicity.

1.8 Responsiveness of Serotonin (5-HT)-2c Receptor following Single Restraint Stress in Rats

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The present study was designed to monitor the responsiveness of 5-hydroxytryptamine (5-HT; serotonin)-2C receptors following the exposure to restraint stress in rats. Rats were restrained for 2 hours. 24-h after the termination of the restraint period, 1-(m-Chlorophenyl) piperazine (m-CPP; a 5-HT-2C agonist) at the doses of 1.5 mg/kg or 3.0 mg/kg and saline (1 ml/kg), was injected to unrestrained and restrained animals. Hypolocomotive effects (home cage activity, open field activity and motor coordination) and anxiogenic-like effects (light-dark activity and plus maze activity) were successively monitored in each animal after the drug or saline administration. Rats were decapitated 1-h post-injection to collect brain samples for neuro chemical estimation by HPLC-EC. Our results show that differences in the hypoloco motive and anxiogenic-like effects of m-CPP between restrained and unrestrained animals could not be demonstrated in the present experimental paradigm. This was due to the fact that the behavioral effects were already marked after restraint leaving little room for an additional m-CPP effect. The effects of m-CPP on increasing 5-hydroxyindole acetic acid (5-HIAA) and decreasing dihydroxy phenyl acetic acid (DOPAC) levels were more pronounced ($p < 0.01$) in restrained than unrestrained animals. The results suggest that behavioral responses to m-CPP and stress are similar The attenuation of m-CPP changes in 5-HIAA and DOPAC levels may be due to a decreased responsiveness of 5-HT-2C receptors in restrained animals.

1.9 Association of Endothelial Nitric Oxide Synthase Gene Polymorphisms in Preeclampsia/ Eclampsia

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Multifactorial, multisystem hypertensive and vascular disorder which is unique to human pregnancy is preeclampsia, potentially life threatening syndrome and clinically manifested as de novo onset hypertension and proteinuria , that resolves postpartum, occurring after 20 weeks' gestation, when severe leads to seizures of eclampsia and it is one of the greatest mysteries in the field of gynecology and obstetrics. The aim of the study was to check the eNOS Glu298Asp polymorphisms in exon 7-T to G conversion at nucleotide position 894 resulting in replacement of glutamic acid with aspartic acid at codon 298 in preeclampsia / eclampsia. As previously no work was performed on this aspect in Pakistan therefore the study holds importance. The blood samples of 30 preeclamptic and 30 healthy pregnant women were collected from different Hospitals. DNA was extracted which was followed by PCR-RFLP and data was analyzed using statistical analysis. The results indicated no association between eNOS gene Glu298Asp polymorphism and preeclampsia. The polymorphism i.e. T to G conversion is still under investigation. The study specified that the most common genotype was G/G homozygous as opposed to T/T genotype which did not exist. Moreover G/T heterozygous genotype was also present. In PE allele T is frequent and in control allele G is frequent. The study concludes that there is no impact of family history and cousin marriages on PE. More research is required to be carry forward the in depth knowledge of the factors related to occurrence of PE with large number of samples. Other genetic factors may contribute to the onset of preeclampsia.

1.10 Inhibitory Effect of Cyanidin-3-Glucoside on Proliferation and Induces Cell Death in Human Prostate Cancer Lncap Cells through Calcium Homeostasis of Endoplasmic Reticulum.

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The level of Ca^{2+} in the within of the endoplasmic reticulum is one of critical factor for cell growth and death. Cyanidin-3-glucoside (C3G) is a member of anthocyanins which belong to the flavonoid family. In this study, we investigated the effects of C3G extract on proliferation, cell death and calcium homeostasis of ER in human prostate cancer LNCaP cells. Treatment with C3G (1 µg/ml to 100 µg/ml) for 24 h - 72 h significantly inhibited cell proliferation in the absence and presence of epidermal growth factor (EGF) (2 ng/ml), in time- and concentration-dependent manner. C3G (1 µg/ml to 100 µg/ml) for 24 h - 72 h also induced cell death in the absence and presence of EGF (2 ng/ml) in time- and concentration-dependent manner. C3G (15 µg/ml, 24 h to 72 h) significantly reduced the increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by treatment of thapsigargin (1 µM, 24 h to 72 h) in the absence and presence of EGF. Under Ca^{2+}-free condition, C3G (15 µg/ml, 24 h to 72 h) also reduced the $[Ca^{2+}]_i$ following treatment of thapsigargin (1 µM, 24 h to 72 h). Treatment with C3G (15 µg/ml, 24 h to 72 h) significantly reduced the Ca^{2+} concentration in the ER in the absence and presence of EGF. Our data suggest that cyanidin-3-glucoside inhibits proliferation and induces cell death in human prostate cancer LNCaP cells through calcium homeostasis of endoplasmic reticulum. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022014)" Rural Development Administration, Republic of Korea.

1.11 Evaluation of Anticancer Potential of Stem Bark Extracts of *Cassia fistula* L.

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Cancer is one of the most life-threatening diseases and serious public health problems in both developed and developing countries. In modern medicines, chemotherapy, radiotherapy and surgery are the major treatments available for cancer. Because of high death rate associated with cancer and because of the serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative and/or complementary methods of treatment. The present study aimed to evaluate the *in vitro* anticancer potential of crude methanolic extract and subsequent solvent fractions of *C. fistula* bark using *A. cepa* root tip assay by keeping them in different concentrations of the test extracts for 48 hours under specific experimental conditions, followed by determination of mitotic index. The results indicated that the mitotic index of *A. cepa* root tips was considerably decreased in comparison to the control in a concentration dependent manner. The lowest mitotic index value was found to be 34% for n-butanol fraction indicating strong antimitotic activity whereas highest mitotic index was for aqueous fraction i.e., 62% indicating a low antimitotic activity. The plant extracts also showed strong antioxidant activity in n-butanol fraction whereas lowest antioxidant activity was observed in n-hexane fraction. Phytochemical screening of plant extract revealed the presence of glycosides, xanthoproteins, tannins, flavonoids, steroids, phenolics, triterpenes, anthroquinones and coumarins while alkaloids were completely absent. The present study therefore, establishes remarkable *in vitro* antimitotic effect of *C. fistula* bark against the test system and indicates the potential of these extracts to be used as a potential source of anticancer compounds.

1.12 Stable Nano-Suspension Formulation for Brain Targeted Drugs to Improve *In Vitro* and *In Vivo* Efficacy

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Epilepsy is the abnormal electrical influx that affects brain functioning in multifaceted manners. Multiple drug resistance takes place at blood brain barrier (BBB) due to overexpression of efflux transporters like p-glycoproteins. NP proved to be a promising agent because they modulate the activity of transporters. Nano-suspension drugs are small enough to cross blood brain barrier coated with antiepileptic drug (AEDs) can be targeted to GABA receptors in the brain which offers improved pharmacokinetics, harnessed and uninterrupted unleash of drugs with much less systemic toxicity in more controllable way. Nanoparticles are quite quarry specific in conjunction with biocompatibility, but hematoencephalic barrier is the preeminent check mark which have to be passed over, AEDs modification, BBB alteration or direct drug delivery can be the difference. Lipophilic molecules can easily cross this hindrance, so liposomes are prime selection for AEDs.Drug release is limited by the desorption and susceptibility to degradation of polymer, by manipulating the factors, AEDs can be maintained for long periods. Delivery systems like Nano suspensions, solid lipid Nano particles or liposomes are prime candidates for the drug delivery.

1.13 Anticancer Activity of The Phytochemicals Obtained from the Methanolic Extract of *Lippia Nodiflora.*

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Lippia nodiflora (Mat Lippia) is the important member of the family verbenaceae showing a variety of medicinal uses. The plant characteristically grows in maritime areas near rivers showing strong preference for wet grassy places. The plant has a long history of reported biological activities particularly as medicinal agent. The aerial parts of the plant are reputed chiefly as carminative, diuretic, antipyretic, emollient, febrifuge and show significant analgesic activity in rodents. In the present studies antitumor activity of the compounds isolated from the methanolic extract of the plant is reported against the full panel of 60 human cell lines.

Some compounds exhibited significant to good cytotoxic activities against different cancer cell lines. Compounds 3, 11-dioxo-urs-12-en-28-oic acidand 3-oxo-urs-12-en-13β, 28-olideshowed promising activity at the aforementioned concentration and illustrated potential against coloncancer cell lines.

1.14 The Genetic Interaction of Draxin and Tsukushi Is Essential For Forebrain Commissure Formation

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Our previous reports documented the identification of two secreted proteins: draxin and Tsukushi (TSK). Draxin is a chemorepulsive axon guidance molecule for a variety of neuron classes and that genetic deletion of draxin in mice results in the absence of all forebrain commissures: corpus callosum (CC), hippocampal commissure (HC), and anterior commissure (AC) (Science 323, 388, 2009). Furthermore, TSK is a member of small leucine rich proteoglycan family (SLRP) and modulators of key morphogens such as BMP and Wnt (Dev Cell 3, 347, 2004; PNAS 108, 14962, 2011). Genetic deletion of TSK in mice also results in the agenesis of AC and CC (BBRC 402, 813, 2010). Because both draxin and TSK single mutant mice converge into common phenotypes—malformation of the CC and agenesis of the AC—we were interested in observing whether these two molecules interact genetically to form the CC and AC. In this study, we generated draxin/TSK doubly heterozygous mice and compared the AC and CC phenotypes of doubly and singly heterozygous mice. The immunohistochemical analysis and DiI labeling showed that compared to singly heterozygous mice, draxin/TSK doubly heterozygous exhibited markedly higher frequencies of AC and CC malformation. We also investigated whether TSK has guidance activities for anterior olfactory and cortical neurons; surprisingly, our results provide the first evidence that TSK has axon guidance activity. TSK binds and inhibits anterior olfactory and cortical neurite outgrowth *in vitro* in a dose dependent fashion. TSK also induces growth cone collapse when added acutely to dissociated neuronal culture. In addition, TSK and draxin have additive effects in reducing neurite outgrowth. Together, these observations establish the contribution of draxin and TSK function to forebrain commissure formation.

1.15 Anticancerous Potential Analysis of Some Plant Extracts against Human Cervical Carcinoma Cell Line

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Cancer, caused by the uncontrolled division of cells, is the second most common cause of deaths worldwide. Due to the side effects associated with treatment through chemotherapy and gamma radiation; there is a need to discover other alternatives. This is now being done through natural sources. The purpose of this study is to explore the anti-cancerous potential of natural plants and fruits as alternatives of synthetic compounds, by using Human Cervical Carcinoma (HeLa) cell line. Aqueous, Ethanolic and Methanolic extracts were obtained through homogenization and microfiltration from various natural sources such as onions (*Allium cepa*), turmeric powder (*Curcuma longa*), rose (petals), grains of pomegranate (*Punicagranatum*), Mushroom (*AgaricusBisporus*), and bitter gourd with skin (*Momordicacharantia*), pumpkin (*Cucurbita maxima)*, beet root (*Beta vulgaris*), dates fruit and leaves(*Phoenix dactylifera*) and American daisy flower and leaves (*Melampodiumpaludosum*). Extracts were serially diluted (up to 64 folds) into 96 microtiter well plates and were screened for their anticancerous potential against the HeLa cell line via the neutral red uptake assay. Results implied that all of the extracts did show antiproliferativeeffects to some extent. However, ethanolic extracts of turmeric and onion and methanolic extract of pumkin and American daisy leaves were the most effective against the HeLa cell line with IC50 at 64 fold dilution of extract. Specific active agents from these extracts can be exploited as potential chemotherapeutic drug targets.

1.16 Curative Aspects of Medicinal Plant Combinations against Isoproterenol Induced Myocardial Infarction

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Myocardial infarction (MI) was induced in rabbits by isoproterenol (85 mg/Kg body wt.). Five indigenous Pakistani plants *Allium sativum, Allium cepa, Zingiber officinale, Terminalia arjuna* and *Moringa oleifera* were selected to prepare 64 herbal combinations. Native as well as gemmomodified plant extracts of selected parts of *Allium sativum, Allium cepa, Zingiber officinale, Terminalia arjuna* and *Moringa oleifera* were prepared and combined to make treatment combinations against isoproterenol induced MI. Both the preventive & curative ways of treatment were employed on different treatment groups prepared by placing 140 healthy rabbits in specific groups. Effects of different plant combinations were calculated through the serum level of biochemical markers at calculated time intervals. Plant combinations decreased the isoproterenol induced rise in CK-MB, LDH, ALT and AST to the level of these parameters in C1 and C2 control groups as compared to C3 (isoproterenol group). The sharp decrease in the level of these enzymes was observed in class III (GP: combination of gemmo extracts used in preventive way) followed by class I (GC: combination of gemmomodified extracts used in curative way). Out of 64 combinations of five plants, 16GP showed the excellent cardioprotective potential by lowering the levels of CK-MB (140.42±7.36), LDH (295.84±17.82 IU/L), AST (43.81±1.16 IU/L) and ALT (49.21±1.42 IU/L) near to the level of these parameters in the control groups.

1.17 Bioassays Application for Cytotoxicity and Mutagenicity Evaluation

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Bioassays based on higher organisms are recognized as excellent genetic models to detect cytogenetic and mutagenic agents in metrics. *Allium cepa* among higher plants is regarded as efficient model for cytotoxicity evaluation, and brine shrimp and heamolytic are also in use for cytotoxicity monitoring. The Ames test is a reference test in chemical mutagenicity testing. In current research, *Allium cepa*, brine shrimp, heamolytic and Ames tests efficiencies were compared for toxicity monitoring of different model compounds simulated solutions and industrial wastewater. Advanced oxidation process (AOP) was used to treat the toxic model compounds and industrial effluents and treatment efficiency was evaluated on the basis of toxicity reduction and improvement of water quality parameters. In response of 90% degradation of surfactant, 70% and 50% reductions in COD and TOC were observed. *A. cepa*, heamolytic and shrimp assays showed cytotoxicity reduction up to 68.65%, 77% and 94%, respectively. The mutagenicity reduced up to 79% (TA98) and 82% (TA100), respectively of surfactants and similar trend was observed for industrial effluents. In conclusion, the bioassays provide a fast, sensitive, reliable and low cost test system to detect cytogenetic and mutagenic agents and are potential alternative candidates to the analytical methods for toxicity evaluation.

1.18 Mutation Induced Enhanced Biosynthesis of Streptokinase

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The bacterial strain *Streptococcus equisimil* is known for production of production of extracellular Streptokinas which is a thrombolytic agent. The strain *S. equisimilis* was subjected to quantitative screening for extracellular streptokinase hyper production. The selected strain was subjected to physical (UV) treatments in order to increase and improve its thermolytic potential. During the treatment, mutants were qualitatively and quantitatively selected and SCGL-UV-19 was found to be the mutant showing highest. Fermentation was carried out in shake flasks and the most potent strain producing 4.20 ± 0.003 U mL-1 of enzyme was selected. The strain was then identified on the basis of standard biochemical and morphological measurements and was assigned the code SCGL-UV-19. Streptokinase production (110.37 ± 0.03 U mL-1) with a zone size of 12.3 mm on heart infusion medium (BHI) was achieved. This mutant showed an overall 338% increase in activity over its parent strain for the production of extracellular streptokinase.

1.19 Prevalence of *mec*A and *luk*S/F-PV gene in *Staphylococcus aureus* isolated from four tertiary care hospitals of Lahore city

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Methicillin resistant *Staphylococcus aureus* (MRSA) causes serious diseases in humans which lead to high mortality and morbidity. Itcontains a toxin called panton valentine leukocidin which destroys leukocytes and mostly associated with skin and soft tissue infections (SSTI). This study was design to find out the prevalence of *mec*A, *luk*S/F-PV genes in*S. aureus*. A total of 376 randomclinical swabs were collected from SSTI (n=179), nasal nares (n=134) and burn wounds (n=63) from four tertiary care hospitals of Lahore during June 2014 – July 2015. The specimens were cultured on blood, nutrient and mannitol salt agar. Plates were incubated at 37°c aerobically. Identification of the organisms was done on the basis of morphology, culture characteristic and confirmedby catalase, coagulase and DNase tests. Antimicrobial susceptibility, MRSA and D-test was determined as per Clinical Laboratory Standard Institute (CLSI) 2014 guidelines. Molecular identification of the *mec*A and *luk*S/F-PV was done using specific primers by polymerase chain reaction. Out of 376 specimens; 127 (33.77%) were *S.* aureus; 41 (32.3%) were MRSA and 86 (67.7%) were MSSA. *mec*A gene was found in 41 MRSA and *luk*S/F-PVgene was found in 21 MRSA and 31 MSSA. Overall, 100% MRSA showed resistance against penicillin and 98% to co-amoxiclav, tetracycline and ciprofloxacin whereas *luk*S/F-PV positive MSSA were 100% resistant to penicillin, 90% to tetracycline and 87% to co-amoxiclav. Inducible clindamycin and high level mupirocin resistance was also observed in 23.6% and 19.5% of isolates respectively. Current study concluded that a continuous and closed surveillance is required at local and national level.

1.20 Airborne Fungal Analysis of Different Units of Hospitals Present In Bhimber, Azad Kashmir Pakistan

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Airborne indoor and outdoor environmental fungi were assessed from six different governmental and private hospitals in Bhimber, Azad Kashmir. This was determined through 'open plate technique'. This was carried out to assess the level of airborne pathogens and to establish standards for further reference. The aerospores of fungi were trapped in petri dishes which have already contained PDA media during April and May from different locations of the selected hospitals. Although a culture-based analysis is most widely used for bio-aerosol, six public hospitals were selected for indoor/outdoor air bio-pollutant measurement. The indoor places included laboratories, bedrooms, X-ray room, doctor rooms and emergency rooms and medicine room. 28 different fungal pathogens were detected from both the environments. Pathogens were generally more dominant in an indoor environment of hospitals. It was also observed that private hospitals have low concentration of pathogens as compared to government hospitals, especially indoor air of the governmental hospital was more contaminated than that of the private hospital in all units. Maximum fungal pathogens were detected in the patient rooms, while minimum pathogens percent were detected in the operating rooms and emergency rooms. The dominant pathogens were Aspergillus spp. (62.9%), Penicillum spp. (54.6%) and Fusarium spp.(60.8%) in the selected area. The time of visit showed higher microbial rates in governmental hospital, while the private hospital was not affected by this factor. The incidence and severity of the identified fungal species, determination techniques and performance results were also summarized and discussed. We concluded that the indoor air quality of hospitals in Bhimber city, especially the governmental hospital, needs more care and surveillance and should be given priority in private hospitals.

1.21 WEE1 Inhibitors Sensitize H3K36me3 Deficient-tumor Cells to Instigate Mitotic Slippage Leading to Programmed Cell Death

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In Multivariate cancer, trimethylated Histone (H3K36me3) is inattentive that can be obliging as therapeutic cure for cancer when synergize with WEE1 inhibitors (AZD1775). WEE1 inhibitors incurably targeted the H3K36me3 deficient-tumor cells because the Ribonucleotide reductase subunit (RRM2) is regulated by H3K36me3 as transcription initiation factor. Secondly, WEE1 reticence reduces the RRM2 through untimely CDK activation which in combination with Cyclin B (B-CDK1 complex) regulate the M-phase of Cell cycle.Eminent level of CDK results in replication stress and prolonged stress causes cell to enter into mitotic phase compellingly without the completion of DNA replication because of dNTP's Scarcity, causes Chromosomesdispersion or uncondensed chromosomes and disorganized spindles in intra-anaphase, consequential Mitotic slippage. The Abnormal nuclear morphology accompanying micronuclei formation results in apoptosis through caspase 3/7 activation or long term cell arrest.

1.22 Study of Prevalence of Cystic Fibrosis among Young Children in Pakistan

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Cystic fibrosis is one of the most common genetic diseases in children and young adults. It is a life-threatening disorder. Cystic fibrosis (CF) is caused by a defective gene which causes the body to produce abnormally bulky and sticky fluid, called mucus. This mucus builds up in the breathing passages of the lungs and in the pancreas. The disease may also affect the sweat glands and a man's reproductive system. It is diagnosed in the children mostly by age of 2 years. A small number, however, are not diagnosed until age 18 or older. The present study was in the context to appraise the prevalence of cystic fibrosis among the young children of Pakistan. A data of 100 patients were collected from the Biocare Labs (Pvt.) Ltd Islamabad. After performing a precise Sweet chloride test on the suspected children .The collected data was subjected to appropriate statistical analysis. It was found that frequency of suspected male children was (63%) whereas frequency of suspected female children was (37%). Out of 100 suspected patients (15%) were reported positive for cystic fibrosis. It was noted that frequency of positive cases lies more in males (57%). Most of the positive cases were reported in the age group of 0-5 years (64%). It was also observed that positive cases were more reported in Khyber Pakhtunkhwa province of Pakistan and the reasons might be a lot of migrants come to this area during war in Afghanistan carrying the defective cystic fibrosis gene.

1.23 Clinical Importance and Molecular Characterization of CTX-M beta-lactamases enzymes

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Beta-lactamases are the notorious enzymes having ability to hydrolyze the beta lactam antimicrobial drugs especially the 3rd and 4th generation cephalosporin and monobactams but not cephamycins due to presence of certain suicide inhibitor like tazobactam, clavulanic acid and sulbactam. Beta-lactam gene encode 291 amino acids and extended spectrum beta-lactamases(ESBLs) are results of one to seven point mutation. Even a single amino acid mutation results in new CTX-M type. Mostly ESBL enzymes were ceftazidimases having little hydrolytic activity against cefotaxime. In 1989 first cefotaximases enzyme were isolated from *E.coli* having maximum hydrolytic activity against cefotaxime and designated as CTX-M. Almost 128 CTX-M types have been discovered so far. CTX-M are classified in five major clusters based upon their amino acid sequences and phylogenetic relationship. Clinically CTX-M are most important due to high hydrolytic activity against cefotaxime "the wonder drugs of time".*Enterobacteriace* is the major carrier of CTX-M genes in hospital settings. Most of the CTX-M belonged to the phylogenetic groups D and B. Many sequence types (ST) or clonal groups are associated with CTX-M. *E. coli* O25:H4, ST131 is one of the high risk international clone associated with extra intestinal infections because presence of various plasmid borne antibiotic genes. *E.coli* harboring ST131 showed maximum resistance with extended spectrum cephalosporin (ESC) and flouroquinolones. While non-ST131 strains show maximum resistance against flouroquinolones and least to ESP, exhibiting the association of CTX-M with ST131 which contributed in resistance against ESP. Global dissemination of CTX-M is said to be related with extensive international traveling and immigration. Animals, animals-derived food and natural environment are also the cause of spread.

1.24 Design and Synthesis of Ibuprofen and Naproxen Derivatives as Cholinesterase Inhibitors via *In vitro* Models

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The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is a capable approach for cure of neurological disorders such as Alzheimer's disease. Alzheimer's disease is the cognitive dysfunction starts with memory impairment leading to dementia common in old age .A series of enantiomerically pure ibuprofen and naproxen derivatives was designed through *in-silico* docking studies, synthesized and screened for *in-vitro* inhibition of AChE and BChE. Majority of the tested compounds showed competent inhibitory activity against AChE and BChE. Among the current series, (S)-N'-(4-(dimethylamino) benzylidene) -2- (6-methoxynaphthalen-2-yl) propanehydrazide (**10c)** exhibited the highest AChE inhibitory activity with IC_{50}= 60.31 ± 0.45µM. Conversely, compounds (*S*)-N'-(2-(4- isobutylphenyl) propanoyl) benzohydrazide (**4e**) and (*S*)-4-chloro-N'-(2-(6-methoxynaphthalen-2- yl) propanoyl) benzohydrazide (**9a**) demonstrated the highest inhibitory activity against BChE with IC_{50}= 38.82±0.24 and 57.71± 0.58 at µM concentrations respectively. The experimental results were found in agreement with the oretical docking studies. These compounds interact with AChE and BChE by making hydrophobic interactions and hydrogen bonding. Energies calculated for these interactions found in the range of -5.63 to -9.65.

1.25 Evaluation of antioxidant effects and inhibitory activity of medicinal plants against lipid peroxidation induced by iron and sodium nitroprusside in mice brain

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The present study compares the protective properties of aqueous extracts of five medicinal plants, *Myristica fragrans, Illicium verum, Curculigo orchioides, Glycyrrhiza glabra*, *Embelia ribes* against lipid per-oxidation in mice brain. The antioxidant activities were analyzed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical assay, lipid per-oxidation assay, metal chelation and total antioxidant activity. The plants extracts exhibited inhibition against thiobarbituric acid reactive species (TBARS) induced by pro-oxidant (10 µM FeSO4) in mice brain. Antioxidant activity of the extracts was investigated by the scavenging of DPPH radical at IC_{50}, 26.60±0.45 µg/ml, 42.41±1.2 µg/ml, 71.14±0.6 µg/ml, 151.50±2.3 µg/ml, 106.30±1.1 µg/ml of *Glycyrrhiza glabra*, *Illicium verum, Curculigo orchioides*, *Myristica fragrans* and *Embelia ribes* respectively. The inhibitory effect and antioxidant activity of under study medicinal plants may be due to presence of higher phenolic contents, free radical scavenging activity, reducing ability and Fe (II) chelating ability. These plants may be used to prevent oxidative stress in brain.

POSTER PRESENTATIONS

TRACK 1

1.1-P Microwave Assisted Extraction of Phenolics from *Allium Cepa*

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Allium sativum is an important medicinal plant belonging to the family *Liliaceae*. Various phytochemicals i.e. phenolics are present in this plant which have medicinal potential. In present research work, cold maceration and microwave assisted extraction of *Allium sativum* was done by using different solvents i.e. methanol, ethanol and n-hexane. For cold maceration, the time period used was 2, 4, 6, 8, 10 and 12 hours. The maximum amount of extract (315mg per gram of plant material) was obtained from the ethanol after 12 hours. So, result indicated that higher the time of maceration, higher will be the amount of extracts. While the maximum amount of phenolics (109.572 µg equivalents of gallic acid) was obtained after 6 hours with methanol. For microwave assisted extraction, the time period used were 30, 60, 90, 120, 150 and 180seconds. Different power levels were also used i.e. 200W, 500W, 800W, 1000W. The maximum yield of plant extract (85mg/g) was found in ethanol at power level 1000W. It was noticed that Ethanol has maximum extraction efficiency for microwave assisted extraction of *Allium sativa* as compared to the other solvents. While the maximum amount of phenolics (207.681µg equivalents of gallic acid) was obtained at 800W power level after 30seconds with methanol. On comparison, it was concluded that the best method in this respect was optimized to be cold maceration rather than the microwave assisted extraction and it gave the higher values of extract.

1.2-P Compositional Analysis and Structural Characterization of Partially Hydrolyzed *Sterculia Urens* Gum

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Sterculia urens (karaya) gum was enzymatically hydrolyzed using cellulose. Using an acid Trifloroacetic acid (TFA) and base Barium hydroxide the gum was also hydrolyzed. Hydrolyzed gum Karaya samples were characterized using Fourier transform infrared spectroscopy, differential scanning calorimetry, thermogravimetric analysis, scanning electron microscope and X-ray diffraction. The monosaccharidal composition was determined by using HPLC. The proximate and mineral compositional was determined. IR spectral analysis suggests that after enzymatic hydrolysis of guar gum there was no major transformation of functional group. Thermal analysis revealed no major change in thermal behavior of hydrolyzed guar gum. It was shown that partial hydrolysis of gum could be achieved by inexpensive and food grade cellulase (*Aspergillus niger*) having commercial importance and utilization as a functional soluble dietary fiber for food industry.

1.3-P Knowledge, Attitude and Practice Regarding Dengue Infection in Karachi, Pakistan

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Dengue virus infection causes significant morbidity and mortality in most tropical and sub tropical countries of the world. Dengue fever is endemic in Pakistan and continuous to be a public health concern. There is a lack of awareness on knowledge, attitudes and practices (KAP) of Pakistan regarding dengue infection. The present study aimed to collect the data of knowledge, attitude and practice regarding dengue infection of resident at Karachi, Pakistan. A cross-sectional study was carried out during October 2014-October 2015, in which pre-structure questionnaire was adopted to determine the knowledge, attitude and practices regarding dengue infection. In 300 respondents are which (150 male, 150 female) were interviewed. Of the 300 people were interviewed, 86.5% male and 88% female regarding dengue infection, 59% people have total awareness about dengue infection. 61.99% of the people had a fair knowledge of dengue infection, but this was associated with appropriate attitude in only 49.29% of participants. Furthermore, only 62.65% of respondent were engaged in positive preventive practices. There is a significance correlation between knowledge and attitude (p=0.006), knowledge and practices (p=0.812). Newspaper and TV were the predominant sources of information about dengue infection. The present study suggests that the good knowledge about dengue infection among residents of Karachi did not convert to approval of preventive measures. It indicates that if people are supplied with accurate knowledge through appropriate channels, they may eventually have good practices in prevention of dengue infection.

1.4-P Comparison of Serum Calcium and Vitamin D Levels in Type 2 Diabetes Mellitus s' Patients and Normal Individuals

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Many chronic diseases have become most serious public health problems, because of their high costs for treatments. Type 2 diabetes mellitus is one of the most dangerous metabolic disorders among these chronic diseases with multifactorial etiology. Previous researches showed that vitamin D and calcium played important to prevent form type 2 diabetes mellitus. Our study was conducted to evaluate the role of vitamin D and calcium in type 2 diabetes mellitus to prevent form it and to improve its complications. Fifty type 2 diabetic patients and 25 normal healthy individuals were selected. Blood samples were collected from each subject and measured HbA1c, fasting and postprandial levels of glucose, serum calcium and vitamin D. Results of the following study showed that type 2 diabetic patients had low levels of vitamin D and calcium than normal healthy individuals. Thus low levels of vitamin D and calcium are associated to the poor glycemic control and prognosis of type 2 diabetes mellitus.

1.5-P Automated Enhancement of Compromised Fingerprint Images

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Forensic Science is a branch of science used for the purposes of law. It provides impartial scientific evidence for use in the courts of law for criminal investigation and trial. A fingerprint in its narrow sense is an impression left by the friction ridges of a human finger on any surface which comes in contact with it. As fingerprints can be identified uniquely so from years it is a popular biometric way of identifying offenders. The recovery of fingerprints from a crime scene is an important procedure of forensic science. Besides, images collected from crime scene usually are of poor quality that's why automatic and reliable extraction of the identifiable points (minutiae) is a difficult task for an automated system. Performance of these systems relies on quality of fingerprints. In order to cope with this issue, system being proposed enhances the image so they can be used further for purpose of identification through an automated system. For enhancement, images are first normalized so that variations in gray levels are reduced. In second step, orientations and frequency of ridges are estimated. These two parameters are used in construction of two dimensional Gabor filter which consists of a sinusoidal plane wave of a particular orientation and frequency, modulated by a Gaussian envelope. A properly tuned Gabor filter preserves the ridge structures and reducing noise, effectively. After this, image is binarized so it consists of only two color values, black for ridges and white for valleys. At the end, skeletonization is applied on fingerprints so that there ridge structure is obtained.

1.6-P *In Vitro* Antimicrobial Study, Phytochemical Screening and Heavy Metals Toxicity of Different Parts of *Ballota Pseudodictamnus*

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The study was done to assess the antimicrobial activities of different parts of *Ballota pseudodictamnus*. These activities were then compared with the class of phytochemicals present in different parts of the plant. The heavy metals toxicity in different parts of the plant was also investigated. The results indicate that in root and stem flavonoids, terpenes and phenols were present in ethanol, chloroform and ethyl acetate fractions. While in leaves flavonoids, terpenes and phenols were present in ethanol, chloroform and *n*-butanol fractions which were the most active inhibiting fractions against microbes in *in-vitro* study. In heavy metals study Chromium was above permissible value in all parts of the plant except leaves. Nickel was observed above WHO limit in root. Cadmium and Lead were found above permissible value in all parts of the plant. The Result concluded that different parts of the plant show different inhibition properties. So each part of plant should be checked for antimicrobial assay separately. It is clear from the results that the plant *Ballota pseudodictamnus* accumulates different phytochemicals and metals in different parts (root, stem and leaves) with miscellaneous concentration.

1.7-P Estimation of Serum Sodium and Potassium Levels in Breast Cancer patients

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Fluctuations in the body fluids and disturbances in blood vessels of electrolyte are a multiple risk factor that results from electrolyte imbalance. Sodium and potassium are the principle electrolytes in extra cellular fluid. Abnormality in this balance results in hypo/hypernatremia causing renal failure and coma and in hypo/hyperkalemia this abnormality causes muscular paralysis and cardiac arrest. In breast cancer patients electrolytes imbalance can lead to even death. This study was designed to investigate the changes in serum sodium and potassium levels and to find if there exists a correlation between them, in breast cancer patients and healthy women. Altogether hundred subjects of 20to 60 years of age were included in this study; fifty were breast cancer females diagnosed patients (confirmed on ultrasound/mammogram) and the remaining fifty were normal females (without breast cancer). Results indicated that that there was no significant association between sodium and potassium levels, neither in breast cancer patients (p value = 0.426) nor in normal women (p-value = 0.310). However, only BMI was found to be significantly associated with sodium levels (p value = 0.005) and potassium levels (p value = 0.048) in breast cancer patients, but not in the normal individuals. This study concludes that in breast cancer patients sodium levels decrease below the normal range but potassium levels either decrease or increase in breast cancer patients, when compared to normal individuals. The present study however, did not show any correlation between sodium and potassium levels.

1.8-P Estimation of Serum Creatinine and Hba1c Levels in Unrecognized Glucometabolic Cardiac Patients

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Serum creatinine concentration in cardiac patients serves as a marker of renal damage. Recent studies have proposed that the association of glycated haemoglobin (HbA1c) with cardiovascular risk may be partially mediated by the development of hypertension. In this study, serum creatinine and HbA1c levels in cardiac patients with unrecognized gluco metabolism were estimated and a correlation between serum creatinine, HbA1c, blood pressure and the duration of cardiac problem was found. Blood samples were collected from 25 cardiac patients (cases) and 25 controls of 30-65 years of age with unrecognized gluco metabolism and undiagnosed renal disturbances. Results indicated a highly significant correlation between serum creatinine and HbA1c levels in controls only (p value = 0.000) with (p value = 0.955) in cases. In cases, serum creatinine had a significant correlation with systolic/ diastolic B.P. and duration of cardiac problem while HbA1c had a significant correlation with salt intake, the type of food consumed and positive family history. The study concluded that a considerable increase in serum creatinine and HbA1c levels increases the risk for CVD.

1.9-P Anti Microbial Properties of Medicinal Plants and Interaction with Synthetic Drugs

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From centuries plants have been used as flavoring agents and preservatives in food. They have also been used as remedy to treat certain disease condition. Active principal components of medicinal plants are responsible for their biological properties. Data regarding anti microbial properties of plants is present in the literature and is proved scientifically. Micro organisms are becoming resistant to many available synthetic drugs. A number of plant extracts has been studied with anti microbial properties in comparison to synthetic drugs including their mode of action and strength of activity. *Allium sativum* extract is effective against the strains of *staphylococcus aureus* and *streptococcus mitis*. Essential oil from *Pelargonium graveolens* shows that small amount inhibits the activity of Bacillus species. Alcoholic extract of *Hyptis martiusii* exhibit inhibitory effect against Methicillin-resistant Staphylococcus aureus (MRSA) strains and E.coli. This extract is more effective than methicillin and gentamicin. Synergistic effects of clove and pomegranate has also been reported. Combination of synthetic drugs and plant extracts show interactions which may be agonistic and antagonistic. Penicillin and terpenes have synergistic effect against E.coli and MRSA whereas thymol and penicillin antagonizes the effect of each other against MRSA strains. Elucidation of this type of interactions is necessary to favor synergism and avoid antagonism. Despite of these favourable associations, these combinations require more studies in vivo so that can be used as pharmacological product for humans.

1.10-P Estimation of serum levels of calcium and inorganic phosphorus in breast cancer patients.

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Breast cancer is a type of cancer which is developed by the formation of a tumor on the breast. This tumor invades and causes different electrolyte imbalance. Present study was designed to measure the serum calcium and inorganic phosphorous (Pi) levels and to check the frequency of hypercalcemia and hypophosphatemia in breast cancer patients. Serum calcium and phosphorous levels of fifty breast cancer women of 18-70 years of age group and fifty healthy women of same age group was measured by using semi automated chemistry analyzer (Humalyzer 3000, Human , Germany). Significant variation in these levels was observed. The mean calcium value in BC patients was higher 9.398 mg/dl than controls which was 8.694 mg/dl. Whereas the mean value of level was lower 4.060 mg/dl in BC patients than controls with 4.456 mg/dl. In this study the frequency of hypercalcemia in BC patients was 10%. Whereas, the frequency of hypophosphatemia in this study was only 2%. Thus it is concluded that there is a significant change in serum calcium and Pi levels in BC patients as the disease progresses. So, this study will be helpful for the clinicians to maintain serum calcium and phosphorous levels in BC patients and also preventing them from further complications. This will also help in designing chemotherapy just by keeping these parameters in mind i.e. it should not contain calcium.

1.11-P Hypolipidemic and Antioxidant Potentials of Fruit Waste

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Hyperlipidemia is a condition that is attributed by increase in lipid levels like triglycerides and cholesterol in blood stream. Defect in any step of fat metabolism, give rise to various ailments like atherosclerosis, coronary heart disease etc. Research is going on in order to find a cost-effective, cheap and safe drug. The present study evaluates the therapeutic potential of ethanolic extract (80%) of *Punica* granatum (pomegranate) and *Citrus* sineus (orange) peels against diet induce hyperlipidemia in rats model. A dose of 400 mg/Kg body weight of both peels extract was given orally to rats for 30 days. The result indicated that both peels extract are able to reduce cholesterol, LDL- cholesterol and increase HDL -cholesterol along with triglycerides levels in serum. The evaluation of enzyme like ALP, AST, ALT and chemicals such as bilirubin levels in serum indicated that the peels also possess hepatoprotective activity.

1.12-P Hepatoprotective Effect of Asparagus Gracilis Extract on Arsenic Induced Testicular Damage in Rats

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In this study, the protective effect of methanolic extract of *Asparagus gracilis* was evaluated against arsenic induced hepatic damage in male Sprague-Dawely (SD) rats. Forty rats were divided into four groups. The first group served as a control. The second group of rats was treated with sodium arsenite at the dose of 40 ppm in drinking water. The third group served as a positive control and received an oral dose of *Asparagus gracilis* extract (150 mg/kg). In the fourth group, *Asparagus gracilis* extract (150 mg/kg) was co-administered orally with arsenic (40 ppm in drinking water). All the treatments were carried out for 60 days. Results of the present study showed that treatment of *Asparagus gracilis* extract reversed the activities of serum marker enzymes and cholesterol profile as depleted with arsenic treatment. Arsenic treatment significantly increased hepatic thiobarbituric acid reactive substance (TBARS) levels whereas, catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GSR) activities were decreased significantly. Lipid peroxidation (LPO) was significantly suppressed and depleted antioxidant defense mechanism was restored by the *Asparagus gracilis* extract treatment. These results suggest that *Asparagus gracilis extract* may be used to ameliorate arsenic induced hepatic toxicity.

1.13-P Use of Lovastatin for Low-Cholesterol Poultry Egg Production: A Comprehensive Study

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During the cholesterol synthesis HMG-CoA reductase (HMGCR) reduces the HMG-CoA to mevalonate, a rate limiting step in cholesterol biosynthesis. Statins act as competitive inhibitors of HMGCR therefore are used to lower the blood cholesterol level in the hypercholesterolemic patients. Lovastatin, one of the most potent statins, was produced from local isolate of wild type *Aspergillus terreus* PU-PCSIR-1 under solid state fermentation (SSF). This study was conducted to optimize different parameters for the production of lovastatin. Maximum yield of lovastatin (2880 mg / kg of biomass) was achieved. The integrity of biotechnologically produced lovastatin was analyzed through high performance liquid chromatography (HPLC) and FT-IR. A comprehensive study was conducted to produce low cholesterol poultry egg by using lovastatin. In this regard 3 groups of layer hens fed with different concentrations of lovastatin, atorvastatin and control groups were investigated. It was observed the a total of upto 35 % cholesterol can be reduced in the case of both lovastatin and atorvastatin. However, liver biopsy examination revealed that use of lovastatin was safer compared to atrovastatin. Based on scientific finding it was concluded that lovastatin can be helpful in developing low cholesterol eggs and doesn't affect on health of layer hens. This bioactive compound can also be used for development of low cholesterol meat.

1.14-P Phytochemical Analysis and Study of Antioxidant and Antidiabetic Effect of *Aloe Vera* Collected from Bhimber Azad Kashmir

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Aloe vera is a succulent plant species and cited as being used as herbal medicine since the beginning of the first century AD. Extracts from *Aloe vera* are widely used in the cosmetics and as alternative medicine to treat various medical problems. In current study *Aloe vera* was collected from six different locations of District Bhimber Azad Kashmir. Their extract in ethanol, methanol and ethyl acetate were prepared and evaluated for the presence of various phytochemicals. Further antioxidant and antidiabetic effect of ethanol extract and gel was also determined. Antioxidant activity was finding by DPPH assay. To evaluate antidiabetic effect of *Aloe vera* gel, patients did not use any medicine were selected and effect was checked for 7 weeks. Plant was cultivated to conserve it for the production of gel and to conduct further studies. It was observed that methanolic extract has very good amount of tannins, terpenoids, quinones and flavonoids respectively. Methanolic extract shows about 60 % radical scavenging activity which might be due to presence of phyto chemicals. It was observed that providing *Aloe vera* gel twice daily bring the values of blood glucose, triglycerides, HDL, LDL and VLDL level towards normal value in most of the patient. These results indicate good anti diabetic potential of *Aloe vera* gel extract with no side effects. These results will further increase the use of locally cultivated *Aloe vera* by local population for the treatment of diabetes. It can be purposed that dietary intake of *Aloe vera* will provide micronutrients to the body that will help scavenge the free radicals and prevent development of hyper lipidemia in individuals.

1.15-P Antioxidant and Antiglycation Potential of Methanolic Extracts of Selected Vegetables

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Epidemiological studies indicate the overwhelming evidence that antioxidant rich diets are associated with a lower risk of numerous degenerative diseases. Current project was designed to evaluate the antiglycation and antioxidant potential of the *Ipomoea batatas*, *Daucus carota*, *Solanum Melongena* and *Brassica rapa rapa.* These are locally available and readily consumed. In this context, optimization of methanolic extraction for the isolation of bioactive compounds was carried out. The biological assays bared a considerable antioxidant potential of selected green materials. Among vegetables, *Solanum Melongena* was proved to be superior while *Brassica rapa rapa* was considered to be inferior for phenolics and flavonoids content. A significant antioxidant activity with least IC_{50} as a result of inhibition of free radical scavenging by DPPH method and maximum reducing capacity was also presented in *Solanum Melongena.* All the samples revealed significant inhibitory effects on early and intermediary glycation products through NBT reduction and Girard T tests at different incubation periods. The selected vegetables also exhibited a considerable inhibition for glucose-induced advanced glycation end-product (AGE) in a dose and time dependent manner in *in vitro* BSA-glucose system. As oxidation and glycation are relevant to diabetic complications the result of current research revealed that plants sources with combined antiglycation and antioxidant properties may offer great therapeutic potential to tailor specific healthy diet for the target population.

1.16-P Biological Activities of Methanolic and Aqueous Extracts of *Cymbopogon Citrates* (Lemon Grass) Against Microbes

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Knowledge of medicinal plants and their use is wide spread and based on a long history of its efficacy. Medicinal plants have long enjoyed their reputation as healing agents. Knowledge of their utilization, dosage, effectiveness, longevity and efficiency is vital for the promotion of herbs and plants as medicinal candidates. *Cymbopogon citrates* (lemon grass) belong to the family *Poaceae* which has long been widely studied with respect to its medicinal importance. Keeping in view the importance of flavonoids and other bioactive components this study focuses on by assessing the biological activities of *Cymbopogon citrates* using different extracts at different temperature treatments. The disc diffusion method was used to assess the antimicrobial potency of the methanolic and aqueous extracts *Cymbopogon citrates*. It was observed that all microbial strains found sensitive (>0.51 activity index) to aqueous extract with the exception of *S. aureus* displayed a higher antimicrobial effect than other microbial species. Similarly aqueous extract of lemon grass had high total phenolic and flavonoid contents as compare to methanolic extracts.The present findings show that *Cymbopogon citrates* aqueous extract at temperature 120 °C had a high biological activities due to temperature treatment effect. The results of this study hint at the potential of *Cymbopogon citrates* as sources of antimicrobial and antioxidant agents.

1.17-P Prevalence of Anemia during Pregnancy in District Vehari, South West Punjab

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Anemia is one of the most commonly encountered medical disorders in females. In most of the cases Anemia is reported during pregnancy. It is more common in developing countries. The aim of study is to compare the prevalence of anemia during pregnancy and normal condition in district Vehari, South west Punjab. For this purpose 100 biochemical analysis reports were taken from Fatima Hospital, district Vehari. The average hemoglobin observed in both was below to 10g/dl while average Total Leukocyte count (TLC) was in normal range. Average Neutrophils in anemic female were 52.3% and in pregnant anemic female were 67.4%. Average Lymphocytes, Monocytes and Eosinophils in normal anemic condition were 26.1%, 9.36% and 3.5% respectively. While in pregnant anemic condition were 26%, 3% and 2% respectively. The average total erythrocyte count was 3.87 million/mm^3 in normal anemic female and 3.38 million/mm^3 in pregnant female. It has been observed that Hemoglobin levels and Red blood cells (RBCs), Packed cell volume (PCV), Mean cell volume (MCV) counts were smaller in pregnant anemic condition than in normal anemic condition.

1.18-P MiR-34a Disclosure in Molcular Cancer Therapeutics: A Journey of Thousand Miles Began

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Mir-34a deposed a promising potential tool cater new diagnostics, prognostics and predictive biomarker in clinical oncologynot only changing the set trends of cancer elixir but also identify major obstacles to shape the possible vision for many years to come.It is a fundamental biological entity redress exclusive set of genes and tumorigenesis pathway reduced cancer cells proliferation, tumor regression, and stemness.While first in class miR34a-mimics accredit a giant step to solve cancer dilemma open up new way to treat cancer in active and reliable way via targeting multiple therapeutic contenders at once and with remarkable delivery technologies proved to be a significant anti-cancer activist. The breakthrough of miR-34a biology lead to series of events that make it critical entity to the development of therapeutic regimens and attain most desirable consideration among Biopharmaceutical companies to design broad pipeline of miRNA based oncology therapies to revolutionized cancer care. The key to triumphant cancer might be in our hand but there is a need of anticipation and harnessing the dynamics to target convict cancer.

1.19-P *In Vitro* Antidiabetic Activity of Stem Bark Extracts of *Erythrina Suberosa* Roxb.

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Diabetes mellitus is an endocrine disease which results due to complete or partial deficiency of insulin. Postprandial glucose level (PPG) is its most serious complication which can be reduced by decreasing glucose absorption. This is achieved by suppressing the carbohydrate hydrolyzing enzymes such as α-amylase. In the present study stem bark extracts of *Erythrina suberosa* Roxb. (Fabaceae) were tested for their α-amylase inhibitory activity that is used as a preliminary test to interpret the antidiabetic activity of plants. Butanol extract of *E. suberosa* exhibited highest α-amylase inhibitory activity (90.38%) with IC_{50} value 0.006 mg/mL at 4 mg/mL which was very close to the inhibitory activity of standard drug acarbose with 92.57% inhibition (IC_{50} 0.005 mg/mL). Since oxidative stress has a significant effect in the causation of diabetes, the antidiabetic activity is mostly related to the antioxidant activity. Thus, the butanol fraction of *E. suberosa* showed strong antioxidant activity of 84.91% (IC_{50} value of 0.01 mg/mL) in DPPH scavenging assay at 4 mg/mL as compared to ascorbic acid which showed 43.45% inhibition (IC_{50} = 0.37 mg/mL) at 1 mg/mL. A strong correlation was observed between α-amylase inhibitory activity and antioxidant activity of the plant. The results of the study clearly indicated that the stem bark of *E. suberosa* can be a good source of compounds with antioxidant and antidiabetic activities.

1.20-P Aristolochic Acid induced Phosphatidylserine Translocation in Erythrocyte Membrane

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Aristolochic Acid, a component of Aristolochia plants, has been shown to cause acute kidney injury, renal aristolochic acid nephropathy, Balkan endemic nephropathy, and urothelial carcinoma. Aristolochic acid nephropathy may be associated with severe anemia. The anemia could theoretically be due to stimulation of eryptosis, the suicidal death of erythrocytes characterized by cell shrinkage and cell membrane scrambling with translocation of phosphatidylserine to the erythrocyte cell membrane surface. Signalling involved in the stimulation of eryptosis include increase of cytosolic Ca^{2+} -activity [Ca^{2+}] and formation of ceramide. Cell volume was estimated from forward scatter, phosphatidylserine-exposure from annexin V binding, $[Ca^{2+}]_I$ from Fluo3 fluorescence, and ceramide abundance from binding of fluorescent antibodies in flow cytometry. A 48 hours exposure to Aristolochic Acid ($\geq$ 75 µg/ml) was followed by a significant decrease of forward scatter and increase of annexin-V-binding. The effects were paralleled by a significant increase of $[Ca^{2+}]_I$ and significantly blunted, but not abrogated by removal of extracellular Ca^{2+}. Aristolochic Acid further significantly increased ceramide abundance. It is concluded that Aristolochic Acid triggers eryptosis, an effect at least in part due to entry of extracellular Ca^{2+} and ceramide formation.

1.21-P Medicinal Potency of Protein and Methanolic Extracts of *Fagonia* Species

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Medicinal plants have long enjoyed their reputation as healing agents. Herbal plants enjoy a very respectable position in the medicinal and health care system especially in developing countries. Knowledge of their utilization, dosage, effectiveness, longevity and efficiency is vital for the promotion of herbs and plant as medicinal candidates. The *Fagonia* species belong to the family *Zygophyllaceae* which has long been widely studied with respect to its medicinal importance. Nowadays, synthetic antibiotics are the primary candidate for the treatment of infectious diseases which has led to bacterial mutations and increased resistivity to antibiotics. The medicinal potential of the *Fagonia* species has recently enjoyed a lot of interest and intellectual scrutiny. This study focuses on the same interest by assessing the antimicrobial potential of *Fagonia* species. The potential agents were extracted by using methanol, buffer and protein extraction methods. The disc diffusion method and the minimum inhibitory concentration technique were used to assess the antimicrobial potency of the methanolic, protein and buffer extracts of both types of *Fagonia* species: *Fagonia arabica* and *Fagonia indica*. It was observed that all microbial strains were sensitive (>7mm inhibition zone) to both plants with the exception of *S. aureus* and *G. lucidum* and that *F. arabica* displayed a higher antimicrobial effect than *F. indica.*The results of this study hint at the potential of *Fagonia* species as sources of healing and medicinal agents.

1.22-P Comparative Efficacy of Triclabendazole, Oxyclozanide and Nitroxynil against Bovine Fasciolosis and Its Effect on Blood Biochemistry.

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A study was conducted in order to determine the comparative efficacy of Triclabendazole, Oxyclozanide and Nitroxynil against bovine Fasciolosis and its effect on various blood parameters. For this purpose, 90 infected animals (n=45 cattles and n=45 buffaloes) were screened. After dividing them randomly in three groups of 30 animals each, drug was administered at recommended dosages and route. Study revealed that Triclabendazole was most effective drug of all three in both cattle and buffaloes with efficacy of 97.92% and 100% respectively; Oxyclozanide was 2nd most effective with efficacy of 96.87% and 97.05% whereas Nitroxynil remained at last with efficacy of 93.47% and 92.15% at 21st day post-medication. In comparison of Blood parameters between healthy and infected animals (cattle's and buffaloes), Significant increase was noted in WBCs (White Blood Cells), whereas, significant decrease was observed in RBCs (Red blood cells), Hb (Haemoglobin), and HCT (Hematocrit) values of infected animals as compared to normal one but no significant increase or decrease was observed in MCHC (Mean corpuscular hemoglobin concentration) values within both animal categories.

1.23-P *Moringa oleifera* As Ameliorative of Heavy Metal Toxicity on Male Reproductive System

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Moringa oleifera, grows world-wide in the tropics and sub-tropics of Asia and Africa. This plant has strong concern because all of its parts are edible and possess high nutritional and medicinal values. Its leaves are an excellent source of antioxidants like vitamin A (four times the amount in carrots), vitamin C (seven times the amount in oranges), vitamin B, calcium (four times the amount in milk), protein (twice the amount in milk), and potassium (three times the amount in bananas). Reproductive toxicity due to heavy metal in male is one of the areas of concern in toxicology today. The toxicity of these metals results in the obstruction of spermatogenesis and steroidogenesis, reduction in the testicular weight/body weight ratio, testosterone level and increase in the activities of oxidative stress. The studies on male reproductive system of mice and rats have proved that antioxidant contents of moringa have enhanced the process of spermatogenesis by increasing the level of testosterone in blood. The extracts and parts of moringa also ameliorated the effects of heavy metals on the reproductive system of lab animals. Due to continuous exposure of human to rising level of metals in water and food, it is recommended that *moringa* may be used as ameliorative as well as to treat the male infertility.

1.24-P Energy Dependent Proteolysis In Archaea

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Archaea, the third domain of life, are genotypically and phenotypically diverse collection of microorganisms which have a mutual evolutionary ancestor. There are four kingdoms of Archeae recognized so far i.e. Crenarchaeota,Euryarchaeota,Nanoarchaeota,Korarchaeota.Proteolysis plays a critical role in regulating proteins, maintaining cell growth and rapidly removing them when they are useless thus avoiding accumulation and chaos inside the cell. Protein turnover in Archaea is regulated by both energy dependent and energy independent proteolysis. Energy dependent proteolysis also known as ATP-dependent proteolysis adjusts mitochondrial biogenesis and eliminates misfolded proteins. The main players of energy dependent proteolysis are HsIUV proteasome, LON protease, ClpAP protease, FtsH protease. ATP-independent protease can either metabolize proteins or regulates protein quality to restock amino acid pool but its major flaw is that it cannot remove misfolded or unwanted proteins from the biological system as compared to energy dependent proteolysis. Energy dependent proteolysis is a more efficient way to conserve energy while inactivating/degrading unwanted protein in Archaea. Energy dependent proteolysis is time taking and accurate mechanism to get rid of or to translocate protein in Archaea.The recycling of obsolete protein and protein quality control is mainly managed by energy dependent proteolysis in Archaea.

1.25-P Toxicological Assessment of Heavy Metals in Honey and Its Vicinity

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Plants, water, soil and samples of bee products were collected from the vicinity of university of Agriculture, Faisalabad to determine the concentration of the five heavy metals such as Cd, Cr, Co, Ni and Pb. Concentration in samples was determined by using atomic absorption spectrophotometer (AAS) after acid digestion through different methods. Concentrations of Cd, Cr, Ni, Co and Pb were higher than the permissible limits in most of the cases in the studied samples which show the level of contamination in their environment. Environmental factors such as, water, soil, plants and honey bee products are natural sources which are facing different anthropogenic interruptions in environment so can be used to detect the toxic materials like heavy metal pollution in their surrounding environment.So for the safety of human being and its environment detection and eradication of these pollution sources is very important and natural products can serve their best for this purpose.

1.26-P Effect of Various Light Sources on Biochemical Parameters of Broilers

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Commercial broilers have been selected for fast growth rate which convert raw ingredients and agriculture byproducts into high quality nutrients for human consumption. The quality of their meat is directly related to their health. For this purpose the effect of various light sources including Incandescent (INC), Fluorescent (FC), Metal Halide (MH) and High-Pressure Sodium (HPS) was checked on biochemical parameters in broilers. In this study 500 broilers of one day old were maintained in clean barn under same management conditions except source of light. The biochemical parameters including hemoglobin, erythrocyte sedimentation rate, cholesterol, glucose level, total protein, Albumin, globulin and A/G ratio was measured. In addition to it blood minerals like calcium, potassium, sodium and magnesium was checked. The birds of MH light group were showing better indicators. Hemoglobin level of the birds of Control, INC, MH, FC and HPS groups' was 11.60, 9.57, 9.50, 9.46, 9.41 mg/dL respectively. Overall, next to control group the health status of the birds pertaining to MH light was better.

1.27-P Cancer vaccines; A novel approach for reduction in cancer incidence

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Nowadays, an alarming increase has been observed in the overall morbidity and mortality due to cancers. Although, currently there are myriad of chemotherapeutic agents available for the treatment of cancers but there are very few possibilities for the prevention of cancers. Under such circumstances, vaccines against cancer offer a very promising approach for the proper control of cancers. During last two decades, tremendous studies have shown very optimistic results about the use of cancer vaccines in laboratory animals; although, unfortunatelydue to fear of development of autoimmunity following cancer vaccination, these have been rarely applied in human cases. But the results of recently conducted studies negate this idea.In fact, it depends upon identification of the tumor specific antigen, which to date account for more than hundreds of different types in different cancers. Upon successful administration in the body, these antigens stimulate the innate (macrophages, natural killers and dendritic cells) and adaptive (CD4+ Thelper type 1 and CD8+ Tcytotoxic cells) immune system of the body to prevent from cancer onset. By using these vaccines, incidence of cancer due to non-mutated, non-viral origin as well as of viral origin may be efficiently reduced. Particularly, this approach seems to be very pragmatic in case of tumors which are formed due to viral infections i.e. controlling hepatocellular carcinoma due to *Hepadenavirus* by using its surface antigen as well as mitigating the cervical cancer incidence by using *Papilloma* virus vaccines.But still this strategy for cancer control is at inchoate stages and much refinement is needed about the proper identification of antigens of tumors as well as formulating a suitable protocol of vaccination.

1.28-P Screening and frequency of HBV, HCV and HIV in intravenous and non intravenous drug users in different areas of Pakistan

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Hepatitis C virus (HCV), Hepatitis B Virus (HBV) and HIV contribute a major public health issue around the world especially in developing countries like Pakistan. In developing countries their source of transmission are the reuse of injections, use of different drugs, sharing of drugs etc. The aim of the present study was to screen HCV, HBV and HIV, The analysis of HBV, HCV and HIV positive serum samples for liver function tests, lipid profile and blood glucose level in intravenous and non intravenous drug users in Rawalpindi and Islamabad. The study design was cross sectional and the duration of the study 06 months. The sample size of the study was consisting of 150 drug users. In this study all intravenous drug users (IVDU's) and non-intravenous drug users (NIVDU's) were included. All individuals those do not use illicit drugs were excluded from the study. We found out that out of total population (N=150) mostly adductors used intravenous drugs 68.66% (n=103) whereas 31.34% (n=47) used non intravenous route. HCV was observed to be strongly associated among intravenous drug users 53.39% (n=55) HCV positives whereas only 31.9% (n=15), non intravenous drug users were found to be HCV positives. HIV was present in 4.874% (n=9) among intravenous drug users while 91.26 (n=94) drug users were found to be negative. The positives cases may be assumed to be due to needle sharing or sexual contact. Only 4.2% (n=2) drug users were found to be HIV positive in non intravenous drug users. Harm reduction program are urgently needed particularly for incarcerated intravenous drug users the sterile syringes/needles through syringes/needles exchange programs for those IVDU's who don't discontinue the injecting drug use.

1.29-P Chain Terminator Based Drugs against Anti-Tumor and Antiviral Treatment: An Innovative Way of Efficient Drug Delivery through Multifunctional Nano-Carriers.

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Artificially synthesized chain terminator based therapeutic drugs have proved their significance in antiviral treatment therapies and are continuously gaining significant importance in shutting the viral replicative machinery down. Nucleoside phosphonate analogues, the key terminators in natural replication process of viruses, put their progressiveness and multiplication to an end inside living entity by all means necessary. With the help of state of the art target specific drug delivery technologies, coupling of nucleoside analogues with nano-particals proved to be an effective way towards target specific delivery as well as to lower the toxic effects. Current in-vitro studies on human tumor xenografted mices confirmed DNA synthesis blockage activity when chain terminator based drugs were operated and delivered via oral as well as polymer based nano-particles acted in response to viral and cancer treatment. Multiple different studies have been conducted on nano based delivery systems of drugs against HIV, HCV, Herpes virus and cancer treatment therapies. Still, cancer treatment and antiviral drug designing demands a great deal of attention and novelty towards radical and cutting edge discoveries regarding this dilemma.

1.30-P *Pistacia Chinensis* Extract Protects Bisphenol A Induced Reproductive Toxicity In Adult Male Sprague-Dawley Rats

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Bisphenol A (BPA) is an environmental toxicant, which is used in various plastic products and food packing materials. It can leech from the walls of container to the food, and enters into the body. BPA has been observed to induce hazardous effects on reproductive health in animals and humans. *Pistacia chinensis* is an antioxidant plant used in the treatment of many diseases. The present study was intended to scrutinize the protective efficacy ofethanolextract of *Pistacia chinensis* against BPA induced damage in rat testes. Forty adult male Sprague-Dawley rats were randomly distributed into four equal groups (n=10/group). Group 1: control vehicle; group 2 (50mg BPA/kg BW/day); group 3 (300mg *Pistacia chinensis*/kg BW/day); group 4 (50mg BPA/kg BW/day and 300mg *Pistacia chinensis*/kg BW/day). All the treatments were carried out for 30 days. BPA treatment resulted in a significant ($p<0.05$) decrease in plasma and intra-testicular testosterone concentrations. BPA treatment induced significant ($p<0.05$) escalation in the thiobarbituric acid reactive substance (TBARS), and marked reduction in protein concentrations, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GSR) activities in testicular tissue of rat. Moreover BPA treatment significantly ($p<0.05$) induced sperm DNA damage. However, *Pistacia chinensis* treatment significantly ($p<0.05$) recovered all the damages caused by BPA.On the basis of these findings, it was concluded that *Pistacia chinensis* extract may be used to ameliorate BPA induced reproductive toxicity.

1.31-P Investigation of the effect of processing and storage on vitamin C in fresh and commercial juices

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Vitamin C is also known as ascorbic acid and has several important functions. It is an essential nutrient and play an important role in the development and maintenance of scar tissue, blood vessels, and cartilage. In this study vitamin C in different commercial and fresh juice samples was investigated. Different brands of commercial juices (Pineapple Country juice, Apple Shezan juice, and Mango Nestle Fruita Vital, Orange Rani), powered samples (Tang Orange, Tang Mango, Tang Lemon, Tang Pineapple) and fresh fruits (Orange, Apple, Pineapple, Lemon, Mango, Watermelon, Melon and Tomato) were collected to determine vitamin C. Redox titration was used to determine the vitamin C content in all samples. Maximum vitamin C was observed in citrus fruit such as in lemon. Moreover it was found that tomato also had a high level of vitamin C. The newly manufactured commercial juices had higher levels of vitamin C, whereas stored fruit juices had lower levels of vitamin C, which is lost during storage and preservation.

1.32-P Chemical Nature of Various Types of Renal Stones in the North West of Punjab: A Meta Analysis

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Renal stones are widespread urological sickness in the whole world. Pakistan has possibly one of the uppermost occurrences of renal stone sickness. The main purpose of this study was to determine the chemical composition of renal stones in District Multan. The study included 200 renal stones attained by surgical process of renal stones patients present in Pakistan. The stones were examined by DiaSys analysis kit. Renal stones were analyzed having different materials e.g. Pure Calcium oxalate stones were present in 80 patients, mixed stones of Uric acid and Calcium oxalate were present in 20 patients. There are also number of mixed stones were obtained having different concentrations. Calcium Oxalate was the most predominant chemical composition in stones. Pure Calcium oxalate stones and Mixed Ca-oxalate and phosphate, were found to be more general in this part of country i.e. 40% and 30% respectively.

1.33-P Antioxidant Properties of Various Fractions of Cell Wall Polysaccharides from Various Parts of *Aerva Javanica*

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The objectives of our study are to characterize the ability of polysaccharides for their antioxidant ability of different parts of *Aerva javanica.* Leaves, branches, stem and roots were subjected to obtain different polysaccharides in solvents of varying polarity such as water, Na_2CO_3, EDTA, 4% KOH, and 14% KOH. Total extractable carbohydrates polysaccharides (%), Linoleic acid reduction capacity, Ferous ion chelating ability, total antioxidant activity by phosphomolybdenum and DPPH assay (g/100g dry extract), Iron chelation assay and hydroxyl radical scavenging assay (%) suggests that polysaccharides of *aerva javanica* possess favorable amount of antioxidants and can be used as a medicine in pharmacological formulations.

1.34-P Effect of phytohormones on micropropagation of *solanum tuberosum* l. Cultivar (favorita), antioxidant potential & HPLC quantification of phenolic acids

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Current research work comprises effect of phytohormones on apical meristem and nodal meristem culture, multiplication of regenerated *Solanum tuberosum* shoots and development of roots from in vitro regenerated shoots. Quantification of major antioxidants such as (TPC, Catalase, SOD, and GSH) and antioxidant activity (DPPH and ABTS) from *Solanum tuberosum*. L cultivar (Favorita) evaluated. Major phenolic acids were identified from methanol extract of in vitro regenerated *Solanum tuberosum* clones by using Reverse phase HPLC. Each stage of micropropagation was performed by the use of different concentrations/combinations of phytohormones with benzyl amino purine, Indole butyric acid and Indole acetic acid. The outcome of apical meristem, nodal meristem with MS medium varied in term of length, percentage of culture survival and number of shoot or roots/explant. Best response of shoot induction was acquired on MS medium containing IAA 2.0mg/L alone, ideal development of shoots from apical meristem was obtained with BAP 2.0mg/L and from nodal meristem with BAP 3.0mg/L, while multiplication of shoots showed good results with combination of BAP 1.0mg/L + IAA 2.0mg/L, IAA 1.0mg/L+ IBA 2.0mg/L and alone with IBA 3.0mg/L and IBA 4.0mg/L. From in vitro regenerated plants,methanol extract was prepared and used for the determination of major antioxidants (TPC, SOD, catalase and GSH) and their antioxidant activity by using DPPH, ABTS and lipid peroxidation assays. Furthermore, methanol extract was analyzing reverse phase HPLC for the identification and quantification of major phenolic acids that showed the presence of 03 different types of phenolics such as quercetin (7.25µg/g), vanillic acid (65.59µg/g) and Chlorogenic acid (41.65µg/g). Our study showed that further isolation and purification of essential bioactive compounds may lead to add valued products along with the new and novel bioactive compound for use in the food and pharmaceutical industries.

1.35-P New Era of Rna Nanotechnology For The Treatment Of Triple Negative Breast Cancer: Nanoscale Solution For A Big Challenge

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Bio-Nanotechnology serves as a change of strategy in the war on cancer. One of the key molecular initiator factor is miR21, the oncogenic miRNA that is recognized to play decisive role in Triple Negative Breast Cancer (TNBC) invasion and metastasis. In TNBC, the furious three genes (ER, PR and HER2) are not endorsed as culprit genes. In recent months, it has confirmed that the systemic delivery of therapeutic miRNA (Anti-miR21) to efficiently and specifically block the hulk like behavior of TNBC tumor growth in in-vivo mice models. The nano-assembly of 15nm therapeutic RNA nanoparticle containing 58nt (nucleotides) pRNA-3WJ as a core body, 8nt complementary sequence to miR21 as seed region and 39nt aptamer for specific targeting plus internalizing of RNA nanoparticle into cancerous cell through receptor mediated endocytosis. These RNA nanoparticles are also found to exhibit RNase resistant potential and are thermodynamically more stable than conventional drugs to bound strongly with tumor cells and repress tumor growth at low doses.

1.36-P *In vivo* Anti-emetic Activity and Total phenolics of *illicium verum* hook. F. Fruit

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The fruit of *Illicium verum* Hook. F. has long been used in China as traditional medicine as well as spice. The selected plant part is investigated for anti-emetic activity. The anti-emetic *in vivo* bioassay was done. Chick model was used for the study. Crude MeOH extract and fractions of *I. verum* fruit were evaluated for anti-emetic activity. Emesis was induced by the oral administration of copper sulphate 50mg/kg body weight. The anti-emetic activity was determined by calculating the mean decrease in number of retching in contrast with those of control. All the extracts were used in 3 concentrations 25mg/kg, 50mg/kg and 75mg/kg. Study was carried out in triplicates. Metoclopramide (150mg/kg) is used as standard anti-emetic drug. Crude MeOH extract was active at only 25mg/kg concentration, its inhibition significant was 58.5 %. Observing the activity at only one concentration, crude extract was subjected to fractionation to see which fraction was the most active and show significant activity. Among all the extract fractions, DCM (F2) fraction showed most significant result in all the concentrations (65.8%, 71.8%, and 72.6%) respectively compared with the reference drug metoclopramide which showed significant inhibition at 54.4%. Also the EtOAc (50mg/kg) showed significant inhibition, 68.0% respectively. In the quantitative phytochemical analysis, the total phenolic content showed highest phenolic content in *n*-Hexane (F1) and lowest in DCM (F2).In HPLC analysis of *n*-Hexane (F1), DCM (F2) and EtOAc (F3) fraction,highest peak was seen in DCM (F2)comparable with the standards of flavonoids. And lowest peak in *n*-Hexane (F1). *n*-Hexane (F1) showed the lowest inhibition %, insignificant in anti-emetic activity. From the observations, it is concluded that *I. verum*has potential anti-emetic activity in which flavonoids and phenolic are actively taking part but some other phytochemicals may have an influence in anti-emetic bioassay.

1.37-P Antioxidant Activity and Phytochemical Characterization of *Viola odorata* l. and *Viola biflora* l.

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Herbs have been the highly esteemed source of medicine. Violaceae is a medicinally important family with 20 genera and about 800 species, and just *Viola* and 17 species in Pakistan.Free radicals cause the oxidation of biomolecules, thus more and more antioxidants become essential these days. In current study, crude extracts of *V. dodrata*and *V. biflora*were evaluatedfor their antioxidant activity at two different concentrations of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay i.e. 0.125 mg/mL and 0.5 mg/mL. Initial screening using 0.125 mg/mL of DPPH, both herbs showed excellent results. At minimum concentration of the extract used i.e. 100 µg/mL, observed activity was 91.08% and 89% for *V. odorata*and *V. biflora*respectively and at highest concentration of 1000 µg/mL observed activity was 98.56% and 95.13% respectively. So calculation of $IC_{50}$was not possible, showing high potential of scavenging of both herbs. Extended further, using DPPH in concentration of 0.5 mg/mL, minimum scavenging capacity was 47.20% at 100 µg/mL of plant extract of *V. odorata* and maximum scavenging percentage was 89.23% at 1000 µg/mL. In this case, at higher concentration of DPPH IC_{50} was possible to calculate only in minimum concentration of the extract used and this value was 47.2%.While in case of *V. biflora* it was 40.49% at minimum concentration and scavenging capacity at maximum concentration was 77.10%. Phytochemical analysis unveiled the presence of some important phytochemicals *i.e.,* Flavonoids, phenolics, triterpenes, tannins and *V. biflora* showed alkaloids also. Colorimetric analysis affirmed presence of flavonoids and phenolics while HPLC and TLC justified their presence. TLC sunlight and UV analysis showed bands of flavonoids. It can be concluded that both herbs show high potential of scavenging at lesser concentration of DPPH.

1.38-P Qualitative and Quantitative Analysis of Flavonoids In Jushanda Ingrediants-A Herbal Cough Medication

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A polyherbal formulation used as traditional remedy for the treatment of different infectious bronchial diseases in Unani System of Medicine in South Asian region and in Pakistan. It is being sold in the market with proprietary name of jushanda. Different plants included in jushanda have been used traditionally in whole world for treatment of infectious diseases separately. In Present investigation some selected joshanda ingredients i.e. fruit of *Ziziphus jujuba,* root of *Glycyrrhiza glabra,* whole herbs of *Mentha piperita,* seeds of *Althea officinalis* and flowers of *Rosa indica* were extracted with methanol. Phytochemical analysis shows the presence of flavonoids and phenolics in all five plants selected for the study of jusanda ingredients, whereas saponins are only present in *M. piperita.* Quantitative analysis was done by Colorimetric and HPLC analysis. The total phenolic content of the crude extracts was determined usning Folin-Ciocalteu reagent and total phenolic content of *Z.jujuba, G. glabra, M. piperita, A. officinalis* and *R.indica* are 0.571, 0.494, 1.210, 0.785, 1.280 GAE mg g^{-1} respectively of dry weight of the extract. The total flavonoid content was determined by Aluminium chloride colorimetric assay and total flavonoid content of joshanda ingredients are 7.6176, 12.0731, 10.6565, 11.5095, 6.6275QE (mg/g) accordingly. Same results were obtained from HPLC analysis that supported the fact. All the selected plants give very sharp peak indicating the presence of flavonoids and phenolics. Quantitative analysis done by both methods colorimetric and HPLC analysis are in agreement about the presence of flavonoids and phenolics. Jushanda ingredients under study showed high DPPH radical scavenging capacity ranges from 57.02 to 94.59 %.

1.39-P Phytochemical Analysis and Evaluation of Entiemetic Activity of Some Plants from Family Asteraceae

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Present study was conducted to phytochemically analyze *Centaurea iberica* Trevir & Spreng, *Carthamus oxicantha* M. Bieb and *Silybum marianum* (Linn.) Gaertn for evaluation of their antiemetic potential against $CuSO_4$ induced emesis. Three fractions: *n*-hexane, aqueous and ethanolic fractions were prepared from Crude methanolic extract. Copper sulphate (25 mg/kg) was used to induce emesis in chicks. Different doses (25, 50, 75mg/kg) of each fraction were tested for their antiemetic properties. Significant inhibition and reduction in retching/min was observed. Maximum inhibition (57.63%) was shown by *n*-Hexane fraction of *Centaurea iberica* at 75mg/kg dose. *Silybum marianum* showed 33.36% inhibition of retching at dose of 75mg/kg while aqueous extract of *Carthamus oxicantha* showed 48.40% inhibition at 25mg/kg. Results were compared with the effect of Metoclopramide (25mg/kg) as a positive control (recommended antiemetic drug available at market) and a negative control (normal saline). The phytochemical analysis showed that all fractions of *C. oxicantha* contained phenolics, saponins, triterpenes and volatile oils while Tannins, proteins, triterpenes and volatile oilswere present in all extracts of *S. marianum* and polar extracts like methanolic extract of *C. iberica* contained tannins as chief secondary metabolites. The results suggest that *n*-Hexane fraction of *C. iberica* have highest antiemetic property among the plants used in this study, which provide for the first time the rationale for its use in Pakistani traditional medicine in the management of emesis.

1.40-P Evaluation of Antioxidant and Hepatoprotective Effect of Polyherbal Ethnoproduct on Gentamicin Induced Hepatotoxicity in Rabbits.

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Despite widespread use of ethno products for treatment of ailments concerning the liver, there is still a dearth of scientific evidence needed to establish their efficacy and safety. Therefore, the purpose of the study conducted was to assess the hepatoprotective and antioxidant effects of a commercial ethnoproduct (*Habb-e-Jigar*), on gentamicin-induced liver damage in rabbits. A total of 30 rabbits were used in the study. Grouping was done as follows: Group I served as the normal control group on routine diet, Group II was on gentamicin (80 mg/kg); Group III was on standard hepatoprotective medication, Silymarin (200 mg/kg). Group IV was on *Habb-e-Jigar* low dose (350 mg/kg body weight orally) and finally Group V was on *Habb-e-Jigar* high dose (700 mg/kg b.w.). Blood was collected on 0, 7th and 14th day respectively. *Habb-e-Jigar* showed high percentages of flavonoid and phenolic components upon phytochemical analysis. Gentamicin induced oxidative stress and liver damage were shown by marked elevation in blood serum levels of AST, ALT, ALP and total bilirubin, Co-administration of *Habb-e-Jigar* with Gentamicin restored the aforementioned liver function parameters in a dose dependent manner. Antimicrobial assays of the polyherbal product revealed significant antimicrobial activity and DNA damage properties. In addition to these, histopathological changes also showed the defensive nature of Habb-*e-Jigar* against gentamicin induced noxious damage to liver tissues. The effect of these tablets appeared to be dose dependent, with more significant improvement in liver function with high dose *Habb-e-Jigar* as compared to low dose.

1.41-P Synergistic Nephroprotective Effect of Vitamin C and Vitamin E against Tobramycin Induced Nephrotoxicity in Rabbits

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Tobramycin is an aminoglycoside, which is a prototype drug. It is used as first line therapy against gram-negative, aerobic and facultative bacteria. Nephrotoxicity is most common adverse effects of aminoglycosides. Vitamins are naturally occurring unflavored antioxidants, although they required in minute quantity but have significant importance. They stimulate growth, metabolism and are involved in energy production mechanism. This study is designed to investigate the protective role of vitamins (C and E) against tobramycin-mediated nephrotoxicity. In the current study albino rabbits were divided into following 5 groups randomly, each group contained 6 animals: Group I was on normal saline and routine feed. Group II (treated control I) received tobramycin (60mg/kg), group III (treated control II) was given tobramycin (60mg/kg) + vitamin C (100mg) orally, Group IV (treated control II) was given tobramycin (60mg/kg) + vitamin E (250mg) and Group V (treated control IV) was given tobramycin + vitamin E + vitamin C. Tobramycin induced nephrotoxicity was evidenced by elevated serum levels of creatinine, BUN and reduced serum level of total protein, albumin, and globulin. The significant nephroprotective effect was observed in animals treated with both vitamin C, vitamin E as compared to that treated with vitamin C and vitamin E alone. Antioxidant stress parameters TOS, TAC, MDA and catalase were also determined to check the oxidative balance. The probable mechanism of nephroprotection could be restoration of observed parameters as evidence by biochemical analysis and oxidative parameters.

1.42-P Effect of Zinc, Iron, Calcium and Magnesium on the Severity of Premenstrual Syndrome in Healthy Females

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Premenstrual syndrome (PMS) has been described as one of the most common disorders in women and effects more that 80% of the women in this world. PMS is characterized by a group of symptoms that usually appear during the luteal phase of menstrual cycle and results in a diverse number of emotional and physical changes. In some females the severity of PMS becomes so much so that they have suicidal thoughts which not only risk their life but their normal activity too. The aim of the study is to understand the relationship between nutrient intake and PMS severity. 30 healthy females were recruited for the current study and the interviews were conducted based on the questionnaires to collect all the relevant information. 4-5 ml venous blood samples were collected 3 to 4 days prior to the menstrual flow. The concentration of zinc, iron, calcium and magnesium in the blood serum samples were analyzed using Atomic Absorption Spectroscopy (AAS). The present study revealed a significant relationship between the intake levels, their subsequent blood concentration levels and severity of PMS symptoms. The severity of PMS symptoms has been found to worsen in majority of the subjects taking less than required daily intake. On the other hand, it was discovered that individuals taking diet rich in these nutrients have much less incidence of anxiety, insomnia, abdominal bloating, fatigue, irritability and depression. However, all these symptoms didn't show any significant relationship between their severity and the body mass index of the participating females. So it can be safely concluded that an optimum intake of zinc, iron, calcium and magnesium can help females avoid the adverse effects of PMS.

1.43-P Assessment of Major Health Risk Factors in Workers from Plastic and Leather Industry

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Leather and plastic industry are major key players towards the economy of Pakistan, however, the well-being of those working in these industries requires more consideration as revealed by the present study. Workers in both of these industries are exposed to a lot of hazardous effluents which are causing severe health threats thus limiting their work output. The aim of the present study was to understand the health risks posed to the leather and plastic industry workers and their relationship with their overall health and recovery from disease. 60 individuals were recruited in the current study, where n=20 for each of the study sub-groups i-e workers from plastic industry, leather industry, and controls. A carefully designed questionnaire was used to extract all relevant information from the study participants and their venous blood was drawn for the biochemical analysis. Results revealed that levels of cholesterol, triglycerides, LDL and alkaline phosphatase were all significantly raised in workers from plastic and leather industries, which correspond positively with the number of years they have been engaged in their respective industries. It was very intriguing to note that the levels of these biomarkers were significantly high among workers in processing and tanning units of the plastic industry, indicating that more protective measures are required for worker in these units. Similar results were obtained when ESR levels were measured and it was found that the elevated ESR levels are consistent with the frequency of contracting an infection and negatively correlate with the recovery duration post infection.

1.44-P Molecular Identification of Aminoacidopathies Causing Intellectual Disability in Children

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Intellectual disability may be caused bythe perturbed levels of hormones, metabolites and enzymes in the body, and are collectively referred as inborn errors of metabolism (IEM).Out of 500 or so IEM around 91such disorders are potentially treatable if diagnosed at an early stage of life. In this study, our aim is to characterize such disorders in Punjabi, Pakistani kids by specifically focusing on disorders ofplasma amino acids (aminoacidopathies). For this work, we are planning to collect blood and urine samples (n=400) from normal healthyschool children (IQ>70) and intellectually low/disabled children (IQ<70) under 10 years of age from Faisalabad and Lahore regions of Pakistan. By analyzing these biofluid samples for changes in the amino acids levels by conventional biochemical screening assays, advanced HPLC, and mass spectrometry assays, we hope to diagnose amino acid disorders like: phenylketonuria, argininosuccinicaciduria, citrullinemia, argininemia, maple syrup urine disease etc. Furthermore, in the complex cases, samples will also be further analyzed by genetic (PCR based) assays for confirming diagnosis. As an output of this study, if these IEM are diagnosed at early stage of life then newborn children will have greater chance to live a healthy life with provision of available therapies to prevent intellectual disability in the suspected children.

1.45-P *In vitro* Hemolytic Activity of Crude Saponins of *Chenopodium album* l. using Human Erythrocytes

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Present study was carried out to reveal the phytochemical profile of the leaves, inflorescence and seeds of *Chenopodium album* L. and to evaluate the *in-vitro* hemolytic activity of crude saponins extracted from the leaves and seeds. At the first step, the methanolic extracts of the plant parts were prepared by maceration technique at room temperature and then saponins were sequentially extracted by using different solvents according to their increasing polarity. Phytochemical analysis of crude extracts revealed the presence of saponins in leaves and seeds while inflorescence was devoid of saponins. Percentage outcomes of the saponins in leaves and seeds with reference to their dry weight were 0.14 and 0.04 % respectively. The hemolytic activity of saponins was checked on human erythrocytes using eight different concentrations (5, 10, 25, 50, 100, 250, 500, 1000 µg/mL) of plant saponins. Results showed that significant hemolysis occurs at higher concentrations and no significant alteration on erythrocyte membrane was observed at low concentrations in both cases i.e. leaves and seeds saponins. Among the tested concentrations, maximum hemolysis was obtained at 1000 µg/mL (93.02 % with leaves saponins and 95.05 % with seeds saponins). Saponins show dose-dependent activity. However, seeds saponins were found more active (HC_{50}=461.19 µg/mL) than leaves (HC_{50}=558.09 µg/mL) with reference to hemolytic activity. It is concluded that saponins extracted from leaves and seeds of *C. album* have hemolytic potential at higher concentrations.

1.46-P Elevated Risk of Developing Heart Diseases among Welders

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Welding is a fabrication or sculptural method to fuse metals together. Since the use of personal protective equipment is a rare practice among welders in our country, they are directly exposed to huge quantities of particulate matter released during this process. Even when air pollution is factored in, welders are exposed to much more ultrafine particles of metals and metal-derived gases on daily basis as compared to workers of other professions. The welders thus suffer from a variety of diseases, the most common complaints being that of lung diseases and metal fume fever. However, this high exposure to particulate matter also poses the risk of developing coronary heart diseases in welders. Previous studies have shown that hypertension, elevated ESR and abnormal lipid profile are the major risk factors and directly linked to developing heart disease. The aim of the present study was designed to evaluate these risk factors in the welders and controls and their relationship with their overall health. A total of 80 individuals were recruited for the current study where n= 40 each for the welders group and control individuals. Results indicated that as low as 5% welders used gloves or other protective equipment while mostly (70%) just used a simple hand-held screen for protection. An alarming trend towards obesity was also found which positively co-related with hypertension as 25% of the welders had high blood pressure. The ESR, triglycerides and LDL were all found elevated among the welders as opposed to the normal controls.

1.47-P Synergistic Nephroprotective Effect of Ceftriaxone And Ascorbic Acid Against Tobramycin Induced Nephrotoxicity In Rabbits

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Tobramycin is an aminoglycoside antibiotic which is widely used against severe bacterial infection. The well documented adverse effect of aminoglycosides is ototoxicity and nephrotoxicity. This study involved evaluation of biochemical, histopathological and antioxidant parameters which showed significant nephroprotective effects against tobramycin induced renal toxicity after treating with ceftriaxone and ascorbic acid. The study was conducted on thirty healthy albino rabbits, divided into five groups equally. Untreated control I was given only normal saline and routine feed. Tobramycin at dose rate of 60mg/kg q 12h was administered intraperitoneally in untreated control I. Treated control I was given tobramycin and ascorbic acid 250mg/kg orally. Treated control II received tobramycin and ceftriaxone 100mg/kg subcutaneously every twelve hours and treated control III administered ascorbic acid, ceftriaxone and tobramycin at same dose rate. Blood samples were taken at seventh and 14th day of treatment. Tobramycin administration resulted in significant nephrotoxicity characterized by marked elevation in BUN, creatinine and TOS level. Co-administration of ceftriaxone and ascorbic acid before administering tobramycin resulted in marked decrease in BUN and creatinine, TOS and MDA levels as well as marked increase in antioxidant health biomarkers including TAC and catalase. The results of biochemical analysis and health biomarkers conferred their strong nephroprotective and antioxidant activity against tobramycin induced renal toxicity. Statistical analysis was performed by applying two way analysis of variance ANOVA and Duncan's multiple range test.

1.48-P Cardio Protective Effects by Bioactive Componenets in *Terminalia arjuna*

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The antioxidants decrease or prevent the oxidation of substrate molecules that can produce free radicals, which start chain reactions and subsequently can cause a large number of cardiovascular diseases in humans. The plant *Terminalia arjuna* commonly known as arjuna, a remarkable tree for its important phytochemicals, belongs to the family combretacea. The active components of arjunabark and leaves are tannins,triterpenoidsaponin (arjunic acid, arjunolic acid, arjungenin, andarjunglycosides). Flavonoids (arjunone, arjunolone, luteolin),gallic acid, ellagic acid, Oligomeric Proanthocyanidines (OPCs), phytosterols, calcium, magnesium, zinc and copper. Antioxidant activity of *Terminalia arjuna* extracts varied considerably depending on the nature of extracting solvents as well as the nature of raw materials to be extracted. The arjuna extracts are found to be quite effective in preventing the oxidation and also effective in cardiotonic, diuretic & in hypercholesterolemia. Its cardioprotective effect has been studied in isoproterenol-induced myocardial ischemia model in rats, rabbits and mice by several authors. Among three *Terminalias*, *Terminalia arjuna* was observed to be the most potent hypolipidaemic agent and it also raised high-density lipocholesterol. Besides hypolipidaemia it also induced partial inhibition of aortic atherosclerosis. Its impact on inflammatory and immunological markers, lipid biosynthesis, platelet aggregation, vascular reactivity and molecular actions in different cells of the cardiovascular system are few of the points which need to bead dressed.

1.49-P In Silico Prediction and Effect of Non-Synonymous SNPs in CREB1 Protein

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cAMP Response Element Binding Protein or CREB is a family of protein that is very extensively studies for its importance in the process of Long term memory (LTM) formation. CREB is a transcription factor that is responsible for the transcription of genes that have CRE (cAMP Response Element) in their promoter region. Non synonymous SNPs are important group of SNPs family which brings structural alteration in protein. In this study, we sorted out SNPs present in coding region of CREB1 gene and also evaluated their impact on structure of protein through computational tools. Genomic data has been retrieved fromEnsembl database and then sorted out by using computational tools including POLYPHEN, MUTPRED, PHD-SNP, SIFT, PROVEAN and I-Mutant. Out of 73 nsSNPs of coding region, 12 missense SNPs have been predicted most deleterious which can change the 3D-structure of protein. CREB-1 is involved in the transcription of genes that are required for the formation of LTM, thus any change in the function of CREB-1 may cause an abnormality in the expression of those genes. Thus these nsSNPs that are marked as deleterious may be involved in the psychophysical cognitive disorders like Alzheimer disease and Huntington disease.

1.50-P Comparative Phenotypic Study of 5-Aminosalicyclic Acid and Sulfamethoxazole for NAT1 Acetylation

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The drugs are metabolized by number of enzymes of phase I and phase II, of which phase II biotransformation reactions are most important. The metabolism of drugs and hydrazine by acetylation detoxify them helping in elimination. The drug-metabolizing enzymes give rise to distinct subgroups in the population that differ in their ability to perform certain drug biotransformation reactions. In present project the comparison of phenotyping was conducted by using two probe drugs sulfamethoxazole and 5-aminosalicyclic acid for a total number of 112 healthy subjects of age greater than twenty years. Blood samples of the human volunteers were collected at 6 hour and 3 hour after administration of SMX (500 mg) and 5-ASA (800 mg) respectively after overnight fasting. The demographic data, biochemical parameters, total protein, creatnine and urea level of the volunteers were also recorded to check their health status, while phenotypic assay was done by HPLC for N acetyltransferase1 (NAT1) acetylation. The distribution of acetylator phenotype in the healthy subjects for sulfamethoxazole showed 68 % (male 67.3 %, female 68.3 %) of the population was fast acetylator while 32 % (male 32.7 %, female 31.7%) of the population was slow acetylator. Similarly the distribution of acetylator phenotype in the healthy subjects for 5-aminosalicyclic acid showed 71% (male 65.4% female 76.7%) of the population was fast acetylator while 28.6 % (male 34.6%, female 23.3%) of the population was slow acetylator. The comparative phenotypic concordance between SMX and 5-ASA was ranged from 80-97 %.

1.51-P Disposition Kinetics and Urinary Excretion of Cefpodoxime in Patients with Impaired Renal Function

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Disposition kinetics and urinary excretion of cefpodoxime will be studied in adult male and female patients with impaired renal function having serum creatinine 3-10 mg/dl and blood urea 100-300 mg/dl. In present study eight experiments will be conducted. The drug, 200 mg of cefpodoximeproxetil, will be given as a single oral dose. Blank plasma and urine samples will be taken before drug administration and then more blood and urine samples will be collected at various time intervals, post medication. Blood samples will be collected in heparinized tubes with the help of indwelling cannula and centrifuged to separate plasma. The plasma and urine drug concentration will be analysed by high performance liquid chromatography (HPLC) method equipped with UV detector. Pharmacokinetic parameters will be measured with concentration versus time data by using computer software program. Depending upon kinetic behaviour of the drug in the body, optimal dosage regimen will be recommended. Urinary excretion will be expressed as cumulative percent of dose excreted in the urine. The data will be analysed through least square regression analysis by using computer software program SPSS-10.

1.52-P Molecular Characterization of Extended Spectrum β-Lactamase (ESBL) producing *Klebsiella pneumoniae* and *Escherichia coli* in Hospital's ICUs of Faisalabad, Pakistan

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Extended spectrum beta-lactamases (ESBL), plasmid mediated enzymes, resistant to extended spectrum cephalosporin (ESC). The snapshot of the situation is greatly linked with *E.coli* and *Klebsiella pneumoniae* involved in nosocomial and urinary tract infections. The aim of the study was to characterize the clinically important ESBL types from ICU of District Faisalabad. Total of 82 clinical samples, hospital visitor, invasive devices and hospital environment, were collected from three tertiary care hospitals of District Faisalabad. Phenotypic confirmation of ESBL producing *E. coli* and *Klebsiella* was done by cultivation on CHROM agar ESBL and double disc synergy test. Molecular detection of *E.coli* and *Klebsiella pneumoniae* was done with PCR. Modified Hodge Test (MHT) was performed for metalo-beta lactamases. Clinically important ESBL genes, $bla_{CTX\text{-}M}$, bla_{TEM}, $bla_{NDM\text{-}1}$, bla_{VIM}, bla_{KPC} and $bla_{OXA\text{-}48}$ were targeted by PCR. Co-resistance with antibiotics other than ESC was checked.30 of the isolates were confirmed to be ESBL producer, 17 *Klebsiella pneumoniae* and 13 *E. coli*. Molecular characterization of the isolates showed CTX-M the most prevalent type (n=19) followed by the TEM (n=7). Cluster analysis of the CTX-M showed CTX-M-1 is the most prevalent (17/19) followed by CTX-M-2 (2/19). Other beta-lactamases were not detected in the study. Antibiotic resistance profiling of the isolates showed maximum (100%) resistance with ampicillin and cefotaxime while least resistance with Tigecycline and imipenem. 27/30 isolates, were resistant against three or more antibiotics classes, found to be multidrug resistant. There is an urgent need of proper hygienic environment in hospitals including surfaces and professionals due to the presence of these superbugs.

1.53-P Urinary Excretion and Renal Clearance of Amoxicillin in female Volunteers after Administration of Augmentin

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Amoxicillin is an acid stable, potent wide spectrum, semi synthetic antibiotic with better absorption through oral route. It is more distributed in body tissue and extracellular fluid having low binding capacity to plasma protein. Amoxicillin impedes the synthesis of bacterial cell wall growth. Excretion through urine is its primary route of elimination from body. Renal clearance and urinary excretion of Amoxicillin was determined in female volunteers among local population from Faisalabad. The urine and blood samples of female volunteers taken at specific intervals were analyzed after oral administration of 625mg tablets of Augmentin. Augmentin is the combination of clavulanic acid and amoxicillin. Before administering the tablets, blank urine and blood samples were taken. The pH of each sample was recorded. The concentration of Amoxicillin and Creatinine in each urine and blood sample was determined by HPLC assay and spectrophotometric assay respectively. Regression analysis was applied on the data. Urinary excretion and renal clearance of unchanged amoxicillin was 52.54 ± 1.68 µg /ml in plasma and 89.95 ± 5.08 µg/ml in urine. A non-significant relationship was determined.

1.54-P Preparation and Properties of Polymeric Nanoparticle-Based Materials for drug delivery applications

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Polymers are playing significant role in numerous applications; however, they may have low thermal, electrical, and mechanical features. In this regard, polymeric nanoparticles (PNPs) have gained considerable research attention. Transition of a polymer from bulk to nanoscale causes change in the bulk-physical properties. Exploration of the routes for the fabrication of PNPs is important to tune the desired features in nanoparticles. Some of the newest findings in this domain have focused on the synthesis of PNPs. The selection of fabrication method depends on number of factors such as particle size, particle size distribution, area of application etc. There is limited literature regarding the development of suitable approach to synthesize PNPs. Designing a technique to obtain PNPs with improved and efficient properties, thus, is a demanding aspect of polymer nanotechnology for potential applications. Biodegradable PNPs have exhibited therapeutic potential for precise drug delivery applications for the cure of cancer and advanced diagnosis. Targeted PNPs have been utilized for efficient transfer of chemotherapies to tumor cells with less damage to the healthy tissues.

1.55-P Urinary Excretion And Renal Clearance Of Clavulanic Acid In Female Volunteers After Oral Administration Of Augmentin

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Clavulanic acid is an inhibitor of β-lactamases what are the responsible enzymes of penicillin resistance. Clavulanic acid which is naturally produced by *Streptomyces clavuligerus* is being formulated commercially in a combination with amoxicillin in "Augmentin".The renal excretion is the main route of elimination for clavulanic acid from body. Renal clearance and urinary excretion of clavulanic acid was studiedin female volunteer among local population from Faisalabad. Blood and urine samples were collected at various time intervals after administration of 750 mg Augmentin orally. The pH of each blood and urine sample was recorded. The concentration of clavulanic acid and creatinine in each sample was determined by using HPLC assay and spectrophotometric assay, respectively. Mean and SEM values of plasma and urine concentration of clavulanic acid was 12.75±1.829 and 56.54±4.9 µg/ml respectively.

1.56-P *Albizia lebbeck* Gum Purification, Modification, Chacterization and use as Drug Binder

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Plant gums are natural polysaccharides widely used in different industries preferred over synthetic ones because of its easy availability, stability, non-toxicity, biodegradability, chemically inert and less cost. *Albizia lebbeck* plant gum used traditionally to accommodate nutritional requirements and for treatment of different ailments. The first step its chemical composition was studied by proximate analysis and carbohydrates content found high then gum was purified by ethanol precipitation and purity yield was 70%. For the micro and macro elemental analysis Laser induced breakdown spectroscopy (LIBS) was performed. Biochemical parameters such as soluble proteins, protease, catalase, peroxidase, amylase and superoxide dismutase was studied and was found in considerable content. Toxicity of selected gum performed by using two mutant strains, *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100 through Ames bacterial reverse-mutation test and gum was not found toxic. Advanced and modern techniques such as scanning electron microscopy (SEM) for the study of surface morphology, Fourier transform infrared (FTIR) for the study of functional groups, Thermo gravimetric analysis (TGA) for the thermal behavior and stability, Differential scanning calorimetry (DSC) to study the function of temperature as to observe the endothermal or exothermal changes with increase in temperature, X-ray diffraction (XRD) for the study of crystalline nature of gum. Drug binding potential of gum was studied by drug delivery system and on the basis of present study it is suggested that Albizia gum a very good source of pharmaceutical drug binder.

1.57-P Hepatogenic Differentiation Potential of UC-ESCs after Serum Pre-Conditioning

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Liver, the second largest organ in human, plays a major role in metabolism and has a potential to regenerate. Liver fibrosis is a wound healing phenomenon that returns equilibrium between the process of scar formation and liver repair. Stem cells are undifferentiated and unspecialized cells capable of developing into differentiated cells with specialized functions. Umbilical cord epithelial stem cells (UCESCs) are magnificent origin of undeveloped stem cells that have the ability to renovate lost tissues. The study was designed to investigate the potential role of UC-ESCs in liver regeneration in vitro. For this purpose the cord samples were processed in bio-safety cabinet II. UC-ESCs at third passage were subjected to treatment with serum from liver fibrotic patients and normal subjects. After preconditioning UC-ESCs were analyzed for improved wound healing and angiogenesis via Immunostaining, ELISA and crystal violet. Estimation of APOX, CAT, SOD and GSH activity in different experimental groups was also done. For the analysis of gene expression RNA isolation, cDNA synthesis, primer designing and RT-PCR were done. In pre-treatment group VEGF levels of injured serum were higher than normal serum levels in the same group whereas in the post-treatment group VEGF levels of injured serum were significantly higher (almost two folds) than normal serum levels in the same group.

1.58-P Review on Current Advancements in Cystic Fibrosis Research and Future Prospects

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Cystic fibrosis (CF) is an acquired disorder of the bodily fluid and sweat organs. It influences for the most parts of lungs, pancreas, liver, insides, sinuses and sex organs. CF causes your bodily fluid to be thick and sticky. The bodily fluid obstructs the lungs, bringing on breathing issues and making it simple for microorganisms to develop. This can prompt issues, for example, rehashed lung contaminations and lung harm. The manifestations and seriousness of CF shift broadly. A few individuals have significant issues from conception. Others have a milder form of the infection that doesn't appear until they are high schoolers or youthful grown-ups. Despite the fact that there is no cure for CF, medications have enhanced enormously lately. Until the 1980s, most passings from CF happened in kids and adolescents. Today, with enhanced medicines, a few individuals who have CF are living into their forties, fifties, or older. Therapy for cystic fibrosis (CF) has advanced amid the previous quite a few years. A lot of this advancement is a direct result of advances in hereditary testing to absolutely distinguish the hidden reason for CF transmembrane controller (CFTR) brokenness. Nonetheless, with more than 1900 transformations that can deliver a flawed CFTR, the administration of CF can remain a test. A few inventive medications as of late affirmed by the Food and Drug Administration, termed hereditary modulators, focus on the basic infection by tweaking the CFTR imperfection. This survey gives doctors a set up basic characterization plan to control their utilization of these medications. The treatment test of 1900 CFTR changes have been rearranged into 6 physiologic classes, each matched with an accessible treatment to offer patients the most useful change. Drug treatment observing, unfavorable impacts, and signs for cessation should likewise be considered. In this review it is examine that cystic fibrosis disorder have no proper cure some therapies are used to control or minimize the chance of severity in patients.

1.59-P Regioselective Synthesis of 2-Bromo-3-Bromomethyl Thiophene Derivatives *via* Palladium (0) Catalyzed Suzuki Cross-Coupling Reactions: As Anti-urease Agents.

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Regioselective functionalizations of halogenated heterocycles play an important role in organic synthesis. Suzuki-Miyaura reactions have become one of the most powerful tools to build carbon-carbon bonds. These reactions have also been widely exploited in the synthesis of natural products, and the design of the pharmaceuticals. We present herein the Suzuki Cross-Coupling reactions of various aryl boronic acids with 2-bromo-3-bromomethyl thiophene. Significant yield of synthesized cross coupled compounds was obtained (52-91 %). The compounds were screened for antiurease activity. The compounds showed potential antiurease activity. It is concluded that the synthesized compounds could be a promising source of therapeutic agents.

1.60-P Genetic and Biochemical Analysis of Biological Risk Factors Causing Cardiovascular Problems in Type 2 Diabetic Patients

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Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, and/or reduced secretion/action of insulin, which leads to several grave complications including cardiovascular problems. The major risk factors for diabetes are genetic susceptibility, physical inactivity, hypertension, obesity, fat/carbohydrate rich diet. About 10% of Pakistani population have diabetes, and 90% of all diabetics have Type 2 DM (T2D), which is a major cause of deaths owing to cardiovascular diseases (CVD) as it increases the risk of CVD related death by 2-4 times. The main reasons for CVD are the elevated level lipids (e.g Triglycerides, Cholesterol, LDLs, VLDLs) and free fatty acids (FFA) and polymorphism in susceptible genes. To prevent CVD in T2D patients, it is necessary to properly manage the circulating FFA according to health needs by increasing physical activity and diet management. Fatty acid profiling in this regard is of vital importance, which will be done in this project by extraction of circulating FFA, their derivatization to fatty acid methyl esters (FAME) and then analyzing them through GC-MS. Polymorphic analysis of susceptible candidate genes/variants will also be done by genetic assays (e.g. PCR, RFLP, TaqManetc) to find variations which are associated with T2D patients having CVD complications. From this study, we hope to develop and validate GC-MS based method for fatty acid profiling along with identification of fatty acid and DNA polymorphism based biomarkers to assess CVD risk in T2D patients.

1.61-P Analysis of Effects of Serum Electrolytes and Organic Parameter Imbalance in Dialysis

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Chronic Renal Failure (CRF) is a public health problem that has serious impact on mental and psychological health of patients undergoing haemodialysis. The aim of this study was to investigate the renal parameters and serum electrolytes in different persons facing kidney problems. The data of 20 patients (50% male and 50% female) with age between 25 to 45 years was collected for the study of serum electrolytes and some organic renal parameters from Nishtar hospital, Ibn-e-seena hospital and Bakhtawar Amin hospital of Multan. Data analyzed by paired sample t-test by SPSS software. Blood urea, Serum creatinine, Potassium ions, chloride ions and sodium ions were analyzed in all the participants. The overall results showed a quantitative increase from normal values of all these parameters. It reveals that renal function is impaired in these people. It is concluded that knowing the patients' preferences for the treatment of kidney disease is necessary to plan appropriate health care services and enhance the quality of care.

1.62-P Bio-synthesis of Silver Nanoparticles using *Ferocactus diguetii* Plant Extract as Reducing Agent for Antimicrobial Activity

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In this research work, silver nanoparticles have been synthesized by using *Ferocactus diguetii* natural plant extract as reducing agent. *Ferocactus diguetii* a species of cactus family, has high medicinal value and rich in polyphenols antioxidant, has been used for reduction of metal ions. Its extraction is simple and cost-effective and it can reduce the silver ions easily without involvement of any external chemical agent. The bio-reduction of silver nanoparticles was analyzed by UV-Vis spectrometer and further characterized by FTIR and SEM as a function of time and concentration. The synthesized silver nanoparticles were uniform and elliptical shape with average diameter 40 nm. These nanoparticles were active against both gram positive and negative bacteria (*Escherichia coli, Staphylococcus aureus*) indicating their broad spectrum antibiotic ability.

1.63-P Formulation and Physical Characterization of Silymarin Emulsions and Suspensions

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Emulsions are thermodynamically stable and clear dispersions consisting of oil, water, surfactants and co-surfactants. They help in the delivery of poorly water soluble drugs. Silymarin is a hydrophobic drug which helps in the treatment of many liver disorders, decrease activity of the tumour promoters, provide protection against genomic injury, increase the hepatocyte protein synthesis and stabilize the mast cells. The aim of this research project was to formulate and evaluate suspensions and emulsions of silymarin to increase its bioavailability. The suspensions were formulated from natural gums (xanthan, accacia etc.) and emulsions were formulated from oils (castor oil, olive oil etc.) using surfactants (tween 80) and co-surfactants (PEG, MEG etc.). The suspensions and emulsions were characterized with respect to pH, conductivity and refractive index. The suspension formulated from xanthan gum and emulsion formulated from PEG, tween 80 and olive oil were most stable. The suspension and emulsion has shown the refractive index 1.436, 1.431 respectively which was very close to oil. An appropriate pH value for formulations (suspension and emulsion) has been observed (6.9). Average conductivity for the formulations (suspension and emulsion) were investigated (0.18ms/cm, 0.0ms/cm) respectively which show the w/o structure of the formulations.

1.64-P Moringa: A Natural Remedy for the Management of Hyperlipidemia

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Hyperlipidemia is one of the most prevalent causes of the death in developing countries including Pakistan. Hyperlipidemia is a condition characterized by elevated level of total lipids in the blood. Several therapeutic agents are available for the management of hyperlipidemia which are generally effective but have many side effects. Alternatively, medicinal plants like *Moringa oliefera* contain substantial amount of potential phytochemicals having beneficial effects in combating hyperlipidemia without causing side effects. The bioactive compounds in the plants that reduces the risk of chronic diseases comprise; phenolics, dietary fiber, phytosterols and other potent antioxidants. So, exploitation of *Moringa oleifera*as a native, cost-effective and nutritious food plant is the best strategy against different diseases. Moringa leaves are rich in polyphenols mainly flavonoid (quercetin-3-glycoside, kaempferol glycosides) and phytosterols especially β-sitosterol which are responsible for its antioxidant properties and lipid lowering effect. Moringa leaves have been shown to inhibit the formation of atherosclerotic plaque and significantly reduced cholesterol and triglycerides levels. Polyphenols present in moringa leaf extract possessed strong free radical scavenging and antioxidant activity which inhibit lipid peroxidation by acting as chain-breaking peroxyl radical scavenger and can protect LDL from oxidation. Polyphenols also possess a variety of other biological activities, such as reduction of plasma lipids due to up-regulation of LDL receptor expression, inhibition of hepatic lipid synthesis and lipoprotein secretion. So, moringa plant can serve as a food source and their extracts, particularly those containing dietary polyphenolic substances, may have potential as "nutraceuticals" for the prevention of such chronic diseases and improving the health of the individuals.

1.65-P A Comparative Study of *Zizyphus mauritiana* and *Acacia arabica* Regarding Their Antidiabetic Activity and Solvent Extraction Based Characterization

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The present study was carried out to investigate the antidiabetic and phytochemical activity of *Zizyphus mauritiana* and *Acacia arabica*. Five extraction solvents used were; 100% methanol, 50% aqueous methanol, 100% ethanol, 50% aqueous ethanol and 100% aqueous. Antidiabetic activity was investigated by inhibiting alpha amylase enzyme through *in-vitro* assay. 50% aqueous ethanol extract of *Z. mauritiana* showed the best results towards the inhibition of alpha amylase at 10,000 ppm and IC_{50} (µg/mL) value 26.28±0.98. 100% ethanol extract of *Acacia arabica* at 10,000ppm showed best results towards inhibiting alpha amylase enzyme and has IC_{50} (µg/mL) value 21.41±0.98. Phyto-chemical assay was performed to investigate the free radical scavenging activity, total flavonides and phenolic contents. Both plants extracts had efficient activities (*Z. mauritiana* has IC_{50} 28.2±0.48 (µg/mL), *A. arabica* showed IC_{50} 39.71±0.1 (µg/mL), total flavanoids (*Z. mauritiana* has 678.24±0.77 g/100g dry weight of plant, *A. arabica* has 934.34±0.89 g/100g dry weight of plants) and total phenolics (*Z. mauritiana* has 146.36±0.81 mg GAE/g. *A. arabica* has 172. 52±0.994 (mg GAE/g) contents at their concentrations. HPLC analysis was also performed which showed presence of some phenolic compounds including feruic acid, 4-hydroxy-3-methoxy benzoic acid, quercetin, vitamin C, sinapic acid etc. Statistical analysis by one way ANOVA also showed significant results.

TRACK 2

MOLECULAR BIOLOGY, GENETIC ENGINEERING AND BIOINFORMATICS

TRACK COMMITTEE

1. **PROF. AMER JAMIL (FOCAL PERSON)**
2. **DR. AFTAB AHMED**
3. **MR. ZAHID MUSHTAQ**

KEYNOTE LECTURES

TRACK 2

K-2.1. INFOMIC-Based Search for Disease Biomarkers

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Ever increasing knowledge in the field of OMICS, including but not limited to, metabolomics, proteomics, transcriptomics and genomics, has potentiated our understanding of the biochemical processes involved in maintenance and regulation of life activities. In general, human physiological disorders are associated with molecular variations that can be examined at different levels of OMICS. These variations can therefore serve as indicators or biomarkers of the physiological changes and hence the corresponding disorders. Finding such biomarkers is necessary to better manage and treat the associate diseases. These biomarkers are also required for early diagnosis and prognosis of the corresponding diseases. The amount of OMICS data currently being accumulated in various data bases is expected to shape up in a computer-biology interface that can be termed as INFOMICS and it suggests that we may soon be able to pin point exact nature and status of a disease with the assistance of the rapidly evolving computer-based information technology. Our group is involved in such studies to find out biomarkers of two diseases, asthma and breast cancer, prevalent in the Bahawalpur region. Using a combination of SDS-PAGE, LC-MS-MS, Time-Resolved Immunofluorometric (TRIF) Back Titration ELISA (bt-ELISA), RT-PCR and DNA Sequencing techniques we have investigated variations in disease-related proteome, transcriptome and genome and have noticed a few molecules and molecular events having the potential of serving as diagnostic and prognostic biomarkers of the two diseases. We hope that the data generated through wet-lab experiments carried out in our lab will be helpful in the development of INFOMIC-based diagnosis and hence the treatment plans to either eradicate or minimize sufferings of the human beings affected with these diseases.

K-2.2. Genome editing with CRISPR-Cas and its applications

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The next generation sequencing technologies have led to rapid progress in genome sequencing of human, plants, animals and prokaryotes. Genome editing tools such as zinc finger proteins, TALENS and most recently CRISPR-Cas9 allows modification of genome *in vivo*, leading to novel insight and applications. Unlike zinc finger and TALENS, CRISPR-Cas system is based on RNA guided recognition of target sequences and therefore is much simpler and cheaper as compared to other genome editing tools. Therefore, CRISPR-Cas9 technology has emerged as a leading technology and declared as "breakthrough of the year 2015" by American Association for Advancement of Science and journal "Science". The most exciting is the use of technology for creating novel traits and correction of genome mutations in a precise manner. This has led to a debate about safety and ethical concerns. The presentation will cover a description of genome editing tools, followed by applications in agricultural biotechnology.

K-2.3. Biotechnology based sustainable insect control strategies

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Arthropod pests destroy about 25% of the world's annual crop production, contribute to the loss of nearly 20% of crop yield. Insect Parasitoids and their associated polydnaviruses are an under-exploited source of valuable biomolecules of relevant interest for insect control. Virulent and host regulation factors used to impair insect host immunity and redirect its physiology in favor of the developing parasitoid progeny represents one of the largest reservoirs of molecular biodiversity of natural molecules with bioinsecticide activity. Genetic engineering and insect transformation technology provide opportunities for the development of insect natural enemies conferring beneficial traits such as pesticide resistance and cold hardiness. The introduction of gene coding for proteinaceous insect toxins (scorpion toxin, mite toxin, trypsin inhibitor), hormones (eclosion hormone, diuretic hormone) or metabolic enzymes (juvenile hormone esterase) into nucleopolyhedroviruses genome are some approaches to increase the killing potential of the virus. Genetic manipulation of *Bacillus thuringiensis* (*Bt*) genes encoding for proteins toxic to insects offers an opportunity to produce genetically modified strains with more potent and transgenic plant expressing Bt toxin. In addition to the *Bt* delta-endotoxin, several proteins that are effective against certain insects such as the vegetative insecticidal proteins (VIP), alpha-endotoxin, a variety of secondary metabolites and proteins of plant origin are amenable to genetic manipulation. Biotechnology can have a positive impact on food security and can contribute to the sustainability of modern agriculture. Insect control strategies that integrate advance knowledge in biotechnology with traditional wisdom and technology will contribute to the sustainability of agriculture.

K-2.4. Nanotheranostics: A New Era of Nanotechnology

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One of the major obstacles using conventional cancer therapies such as radiotherapy or chemotherapy is the lack of specificity for cancer cells. There is an immense entail for novel therapeutic strategies capable of delivering chemical agents and other therapeutic materials specifically to tumour locations limiting the doses. This may be achieved using nanoparticles (NPs) containing a therapeutic agent. Tumour selective drug accumulation is caused by enhanced EPR effect permitting NPs to cross tumour capillary walls, but not capillaries in normal tissue. Advance imaging techniques for NPs delivery monitoring has enabled real-time assessment of the therapeutic process. Nanotechnology has started a new era in engineering multifunctional NPs for improved cancer diagnosis and therapy simultaneously, Nanotheranostics. We, at NORI, aims at developing these systems to fight against deadly disease of cancer.

K-2.5. Genetically Modified (GM) Crops: A case study shows that "a fungal transcription factor gene is expressed in plants and improves drought tolerance"

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Genetically modified (GM) crops are those in which their DNA modified by using molecular biology/genetics tools involving bacteria and virus, in a way that does not occur naturally. Individual genes across the species can be introduced which can improve the shelf life time, taste and/or nutritional value of plants which are referred as "biologically robust plants". Initially it was started to address the growing concern about protecting crops from insects, climate change and harmful pesticides but as the technology advanced, the concerns about the use of GM crops also rises. We present here the heterologous expression of a fungal transcription factor gene improves drought tolerance in plants as a case study for transgenic technology.

ORAL PRESENTATIONS

TRACK 2

2.1. Construction, Overexpression and Purification of Novel Thymosin α 1 Fusion Protein for Targeted Cancer Therapy

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Thymosin α 1 (Tα 1) has wide variety of therapeutic applications and is currently being used either alone or in combination therapy for the treatment of several diseases such as cancer. It induce the production and differentiation of lymphocytes, increase $CD3^+$, $CD4^+$ and $CD8^+$ cell proliferation, stimulating the production of different cytokines, increasing the expression of IL-2 receptors, recruiting the pre natural killer cells and increasing the production of antibodies. Azurin is a cuperodoxin protein of *Pseudomonas aeruginosa* whose peptide fragment from amino acids 50-77 (*Azu p28*) acts as a potential cell penetrating peptide and preferentially penetrates cancerous cells, stabilizes p53 inside the tumor cells and induces apoptosis through Bax mediated cytochrome c release from mitochondria. In this study, gene encoding Azu p28 was fused with Tα1gene to enhance the anti-cancer effect of Tα 1 and targeting Tα 1 to cancerous tissues. Overlap extension PCR was used to fuse Tα 1 gene and Azu p28 gene and fused gene was cloned into pTZ57/R vector. Transformed clones were screened by colony PCR and restriction analysis. Fusion gene was sub-cloned into pET28 a (+) and recombinant fusion protein was expressed in *E. coli* BL21 (DE3) after 4 hour induction with 1mM IPTG at 37 ^oC. The Fusion protein was purified Nickel chromatography using 150 mM imidazole and characterized with western blot and immune dot blot assays. It is concluded that synergic effect of Tα1and Azu p28 will not only make it more effective anti-cancer drug but tumor penetrating ability of 1Azu p28 will make it targeted therapeutic tool for safe and secure treatment of patients.

2.2. Interaction and Replication of ssDNA Viruses and their associated Satellites

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This study was designed to investigate the diversity of genomic components of begomoviruses associated with a non-cultivated host *Eclipta prostrata*. Leaf samples showing typical vein yellowing symptoms were collected from the different districts of the Punjab, Pakistan. Amplification of the full-length viral molecules and their associated satellite molecules was done through polymerase chain reaction (PCR) and rolling circle amplification (RCA). Cloning of the full-length viral molecules, sequencing, recombination analysis and phylogenetic studies using different softwares like MEGA 5 and DNA star were carried out. These analyses showed that this weed plant carries *Alternanthera yellow vein virus* (AlYVV), along with two different types of Alphasatellite molecules i.e., Alternanthera yellow vein alphasatellite and chili leaf curl alphasatellite. AlYVA showed 83% sequence homology to Mesta yellow vein virus so it was proposed as new species of alphasatellite. There was no betasatellite molecule was found to be associated with vein yellowing disease of *E.prostrata*. Sequence analysis showed that AlYVV is recombination free in all plant samples, which we analyzed. But an interesting feature of alphasatellite on *E. prostrata* was recombination among Malvaceous and non-Malvaceous alphasatellites. The interaction study of alphasatellites with the helper viral for the onset of disease was also studied. Infectious molecules of the full-length viruses and satellite molecules were constructed for infectivity analysis to fulfill the Koch's postulate. Infectivity analysis showed that AlYVV is infectious to *Nicotiana benthamiana* plants and viral movement was confirmed through Southern blot analysis. When alphasatellites were coinoculted with AlYVV, the movement of alphasatellites was confirmed in systemic leaves through PCR.

2.3. Molecular Screening of Buffalo Mycoplasmosis in Karachi, Pakistan

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Mycoplasmosis is a most serious and economically most costly disease of cattle and buffaloes due to its high morbidity and mortality. This type of infections most of the time persist due to mismanagement and unhygienic condition of the farm. Therefore, 112 animals were examined clinically, whereas 49 were observed in respiratory distress (nasal discharge, coughing, sneezing and dyspnea). On the other hand randomly 138 lung samples (slaughtered animals) were studied at abattoir of Landhi buffalo, Karachi. 62 *(45%)* lesions (pnemonic lung) were observed. Of the 62, 39 (63%) samples were found culture positive. These cultures were confirmed by PCR and 21/39 (54%) isolates were found as *M. bovis*. However, all the isolates were found *Mycoplasma* using universal Mycoplasma primer.

2.4. Investigation of genetic control of acetylation, methylation, phosphorylation and *O*-glycosylation

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Bioinformatics is an emerging field and it has become essential to use bioinformatics as a helping tool to perform experimental studies. Genetic control of acetylation, methylation, phosphorylation and *O*-glycosylation of mammalian proteins are investigated *in silico* with the help of bioinformatics tools. Acetylation, methylation, phosphorylation and *O*-glycosylation of mammalian proteins include structural and functional changes that are most often transitory and difficult to follow. *In silico* prediction procedure for these PTMs are valuable to force for disease prognosis and treatment. In this study genetic control of Thr (phosphorylated/glycosylated) and Lys (acetylated/methylated) codons are investigated. The tools used for these analyses are dbPTM, Uniprot, SMS codon plot, Ying O Yang, PAIL, MeMo. These databases provide rich, consistent and non-redundant protein information by combining reliable automated annotation approaches with literature-based expert manual curation. The prediction results suggest that phosphorylation and glycosylation on the four different codons of Thr and methylation and acetylation on the two codons of Lys are not dependent on their respective codons. It was found that PTMs may not be regulated on genomic level but on proteomic level as vicinal amino acids may be determining factors in whether an amino acid becomes acetylated and/or methylated, phosphorylated and/or glycosylated.

2.5. Evaluation of differentially expressed proteins of *Ricinus communis* by SDS-PAGE analysis

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Plants are equipped with biochemical machinery required for the protection of plants from living disturbances. Although plants have their own natural defense system against invading pathogens, however, natural resistance is not enough. Therefore, enhancers must be found for the protection of plants from biotic attack. *Fusarium* species in this respect has the ability to boost up host defense response. In the present study *Ricinus communis* was subjected to fungal induction followed by extraction of total proteins from the plant at different time points. Protein variability among different time points was analysed by SDS-PAGE electrophoresis. Electrophorogram for each time point was scored and presence or absence of each band was noted and entered in binary data matrix. Based on electrophoresis band spectra, differential expression was evaluated by constructing the dendrogram for high molecular weight and low molecular weight proteins. Out of 21 total proteins ranging from 75-8 kDa, 14 proteins were differentially expressed at different time points. A consistent increase in protein expression was observed after inoculation with *Fusarium solani* up to 8 hours post inoculation (hpi) which declined during subsequent hours. It is due to the rapid activation of plant defense proteins/peptides during early hours of induction. Thus, *Ricinus communis* has been found to be rich in peptides involved in plant growth and development, signal transduction and exclusively to the peptides involved in defense response.

2.6. Molecular Studies on Preproinsulin gene

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Insulin plays an important role in maintaining the blood glucose level of the body. The β-cells of pancreas produce insulin in form of precursor that is preproinsulin. The gene of preproinsulin provides an interesting system for addressing question related to molecular evolution. Recombinant DNA technology has made it possible to isolate and sequence the chromosomal genes coding for unique protein products. Although preproinsulin of various organism has been isolated and cloned, but there is no report from buffalo *(Bubalus bubalis)* that is our major livestock. The genomic DNA of buffalo was isolated using Laura-Lee-Boodram method. The part of preproinsulin gene (596 bp and 520 bp) using BPPI-UPS and bpiful_F as forward and BC1-C as reverse primer was amplified. Cloning of amplified fragments of gene were performed in pCR 2.1 vector. Positive clones were screened on the basis of blue white selection. The band obtained on 596 bp and 520 bp after colony PCR confirmed the successful cloning of preproinsulin gene in pCR 2.1 vector.

2.7. CRISPR/Cas9 Based Site Specific Disruption of *PDS* Gene in *Nicotiana benthamiana* Using Transient Gene Expression

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Site specific genome engineering has emerged as a powerful tool to substitute the classical and conventional approaches to develop transgenics. Based on Clustered Regularly Interspersed Short Palindromic Repeats/CRISPR associated (CRISPR/Cas) system, engineered Cas9:gRNA complex can be used to cleave specific DNA sequences resulting in genome alterations. In this study *Phytoene Desaturase* gene of *Nicotiana benthamiana* (*NbPDS*) was disrupted using transient expression of plant codon optimized Cas9 (pcoCas9) and a guided RNA (gRNA) containing 20 nucleotides of *NbPDS* gene. PcoCas9 and gRNA were cloned in pGreen0029 plant expression vector to target *NbPDS* gene. *Agrobacterium* containing pcoCas9 and gRNA cassettes (separate and combined) were introduced in *N. benthamiana* leaves using *agro*-infiltration method. Visible albino spots were observed on *agro*-infiltrated leaves of *N. benthamiana* plants after 7 days of infiltration. These observed spots were subjected to PCR amplification of gRNA-target, fluorescent microscopy and chlorophyll contents measurement which showed that albino spots were due to dis-functioning of *NbPDS* gene when compared with the control plants.

2.8. Saline-stress induced antibacterial cationic peptides from *Solanum lycopersicum* (tomato)

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Development of antibiotic resistance in pathogens has alarmed the exploration of new antibiotics. Cationic peptides are natural antibiotics and play a fundamental role in recruiting and promoting agents of innate as well as adaptive immune system. These are produced constitutively as well as under the influence of biotic and abiotic stresses. In the present study *Solanum lycopersicum* (tomato) was induced to express cationic proteins/ peptides under salt stress. Proteins up to molecular mass of 10 kDa were isolated and fractionated by cation exchange chromatography followed by antibacterial assay. The results showed that the tomato plants could tolerate salt stress of 200 mM for 30 days while 100 mM of stress provoked more antibacterial peptides/ proteins. Ultra-fast liquid chromatographic spectra of the control and the salt stressed tomato plant extracts has depicted that expression of positively charged peptides increased under 100 mM of salt stressed conditions. The strong cation exchange column fractionations of tomato plants under control and salt stress of 100 mM concentration showed high percentage of antibacterial activity against *E. coli*. Antibacterial activities of fractions showed that bioactive cationic peptides produced constitutively (under control conditions) and under the influence of abiotic stress (under salt stress conditions).

2.9. Characterization of Recombinant Thermostable Phytase from *Thermotoga naphthophila* and its Biological Evaluation in Broiler Chicks

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The present study deals with the production and characterization of recombinant thermostable phytase and its biological evaluation in the broiler chicks. The PCR resulted in the amplification of 1.8 kb phytase gene using the genomic DNA of *Thermotoga naphthophila* as template. The purified PCR product was ligated in pTZ57R/T and the ligated material was utilized for the transformation of *E.coli* DH5α cells. The restriction digestion of plasmid DNA from positive clones using *Nde*I and *Hin*d III resulted in the release of insert from the vector. The purified phytase gene after restriction digestion was ligated into pET21a already restricted with the same restriction enzymes and the expression was analyzed using *E.coli* BL21 CodonPlus (DEL) cells. SDS-PAGE demonstrated the intra-cellular production of recombinant phytase. The conditions were optimized for the optimal production of recombinant phytase (PHY_{TN}). The maximal production of PHY_{TN} was recorded when the BL21 CodonPlus cells having recombinant phytase gene were induced with 1.4 mM IPTG and 6 hours post induction incubation period. The purified recombinant protein was utilized for characterization. PHY_{TN} showed optimal activity at 80^0C and pH 6 in sodium acetate buffer. The presence of Fe^{3+} or Cu^{2+} showed enhancing effect on PHY_{TN} activity. Thermostability studies demonstrated that PHY_{TN} retains 90% residual activity when the protein was incubated at 80^0C for 1h in the presence of 1.5 mM Fe^{3+}. The kinetic studies of PHY_{TN} demonstrated K_m and V_{max} values of 50 mM and 2500 µmole/min respectively when sodium phytate was used as substrate. The characterized PHY_{TN} was used for poultry trials to check the efficacy of the enzyme in poultry birds. The results depicted that PHY_{TN} put a significant effect on the bird weight gain, feed intake and feed efficiency ratio. The study demonstrated that this recombinant thermostable phytase is suitable for poultry feed industry and its domestic production will contribute the economic availability of PHY_{TN} for the poultry feed industry.

2.10. Use of DNA nano-biosensors for the detection and quantification of economically important DNA viruses

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Agriculture is the backbone of Pakistan's economy and cotton is a major cash crop. Cotton production is mainly affected by cotton leaf curl disease (CLCuD) which is caused by a complex of viruses that belong to genera *Begomovirus* of family *Geminiviridae*. CLCuD complex is associated with many begomoviruses, but *Cotton leaf curl Khokhran virus*-Burewala (CLCuKoV-Bur) is the most dominant one. Here we have designed carbon nanotube based lateral flow biosensor and electrochemical technique for the fast detection and quantification of CLCuKoV-Bur. For this purpose, carbon nanotubes (CNT) and copper (Cu) composite has been prepared and attached to DNA probe specific to CLCuKoV-Bur. AFM and SEM revealed that nano structure of composite increased the electro-active surface area for the detection of target DNA molecules. Interestingly, designed electrode can detect up to $10x10^{-18}$ (atto gram) DNA in comparison to qPCR (highly sensitive commercially available technique) that can detect up to $10x10^{-15}$ (femto gram). In addition, amine modified bioconjugate with CNT were designed to fabricate lateral flow biosensor for the detection of CLCuKoV-Bur in infected plant samples**.** The test and control probes depicted distinctive black bands demonstrating DNA hybridization reaction. In this way two different types of DNA nano biosensors were designed having higher affectability than other available techniques.

2.11. Development of an Efficient Regeneration Protocol for Wild Type Tobacco

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Commercially grown tobacco *Nicotiana tabacum* L. has a morphologically distinct relative *Nicotiana plumbaginifolia*, wildly grown in Punjab and Khyber Pakhtunkhwa. Wild relatives are primary source of variable genetic material bestowing valuable traits to cultivated plants. Hence, concerted efforts are needed to conserve these species through conventional as well as modern techniques including tissue culture and genetic manipulations. Here, we report a proficient and reproducible *in vitro* regeneration system for *Nicotiana plumbaginifolia* producing multiple shoots per leaf segment. Shoot segments from field grown plants were surface sterilized and cultured *in vitro* in order to maintain contamination free explant source throughout the study. Initially, a number of previously reported tissue culture protocols were examined for their reproducibility. Based upon the results obtained, *in vitro* regeneration methodology was devised employing a variety of growth regulators. The best results were obtained while culturing leaves of *in vitro* grown plants on MS medium augmented with 1mg/L kinetin and 0.25mg/L IAA. Individual shoots were placed on MS medium with/without IBA for rooting. Individual shoots were successfully rooted on MS medium without IBA and plants were grown in greenhouse and field. The protocol reported here will pave the way towards biotechnological interventions of *Nicotiana plumbaginifolia*.

2.12. *Azospirillum* spp. to enhance cotton growth

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Azospirillum spp. play an important role to increase plant growth and productivity. *Azospirillum* spp. were isolated from wheat rhizosphere and characterized and identified by colony and cell morphology, BOX-PCR and 16S rRNA genes sequence analysis. Isolates formed a 3-5mm thick wheel like white pellicle in semisolid Nitrogen Free Malate Medium. They were gram negative, 3-12 µm long plump rod with 1mm in diameter, fast and helically motile under light microscope. Box PCR finger printing was carried out to differentiate the isolates. Box PCR suggested at least 8 different types of *Azospirillum* isolates in the wheat rhizosphere. *Azospirillum* isolates were inoculated to cotton plants after 4 weeks. Single strain inoculation increased the plant height of cotton. Maximum height 47 cm was observed in Azo 16. They have shown potential to improve the growth of cotton plants.

2.13. Identification and Characterization of *ATWBC7* and *ATCNGC19* for their Roles During Abiotic Stress Tolerance in *Arabidopsis thaliana*

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A new approach based on activation tagging was developed for identification of salt responsive mutants in *Arabidopsis*. Using this new method we identified 18 potential mutants resistant to 150 and 200 mM NaCl stress. A salt tolerant callus 5 (*stc5)* and salt tolerant callus 13 (*stc13*) were tolerant to both 150 and 200 mM NaCl. TAIL-PCR analysis confirmed three insertions in *stc5*, one located on chromosome 2 and two others on chromosome 5. While in s*tc13* two insertions were confirmed and both were located on chromosome 3. Real-time PCR analysis of *stc5*, revealed a gene responsible for ABC transporter (*AtWBC7*) and a gene coding cyclonucleotide gated ion channel (*AtCNGC19*) was activated in *stc13*. Knock-out (KO) line of *AtWBC7* was sensitive to 100 mM NaCl stress and also to KCl, KNO_3 and LiCl stresses. On the other hand, KO lines of *AtCNGC19* and *AtCNGC20* did not show sensitive phenotype under salt stress conditions. Moreover, over-expression lines of *AtCNGC19* and *AtCNGC20* also did not show stress tolerant phenotype. Presently, we have been developing over-expression lines of *AtWBC7* and double mutant of *AtCNGC19* and *AtCNGC20* through CRISPR/Cas9.

2.14 *Meta-analysis of miRNAs to identify biomarkers for oral Cancers*

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Oral squamous cell carcinoma (OSCC) is one of the most common cancers worldwide. According to a validated report of registry of Shaukat Khanum Memorial Hospital, Internationally, it is rated at 8th common type of cancer, however, in Pakistan it is the 2nd most common type of malignancy. Recent studies have highlighted the role of miRNA in disease pathology, indicating its potential use in an early diagnostic tool. miRNAs play important roles in modifying various cellular processes. Disregulated expression of miRNAs is known to affect the cell growth, and this may function as tumor suppressors or oncogenes in various cancers.

The main objectives of this study were to characterize the extracellular miRNAs involved in oral cancer (OC) to assist early detection of cancer. We used gene expression data by microarrays already available in literature. miRNAs and their target genes were predicted. Simultaneously, the differentially expressed genes (DEGs) of oral carcinoma from all experiments were identified. The common genes between lists of DEGs of OC based on experimentally proven data and target genes of each miRNA were identified. We performed Molecular dynamic simulations to mimic the behavior of miRNAs in natural body environment. We included some of pathways in cancer to show the change in gene expression under the control of specific miRNA. We observed that a selected set of genes regulated by maximum number of miRNAs, and thereby, they can be used as biomarkers of OC.

2.15. ELISA for transgenic rice plants, insects and predator in tri-trophic interaction

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Transgenic crops were developed to show resistance against the target chewing insects in transgenic crops. Genetically modified rice was also developed to show the resistance to its major insects pests such as Lepidopteran in paddy rice. However some non-target effects were found from genetically modified crops. *Stenchaetothrips biformis*, the most important rice seedling insect pests and one of nontarget insect species of *Bt* rice. In this experiment ELISA was performed for three *Bt* rice lines including H2A, H1C, E1 (expressing the Cry2A, Cry1C and Cry1Ab/Vip3H protein respectively) and their non-*Bt* correspondent parental cultivars HCK and Xiushui 110 under laboratory conditions. For thrips and predator *Orius sp.* ELISA was performed to observe the uptake of *Bt* toxin from transgenic plants to insects and ultimately upto third trophic level. Although sucking insects have no specific site of action of Bt toxin in digestive system. A leaf dip bioassay was carried using the leaf from control cultivar. Different concentration of Cry2A, Cry1C and Cry1Ab proteins in bioassay to observe the effects on mortality of *S. biformis* at different levels was also conducted. Higher levels of *Bt* protein were detected in case of H2A (Cry2A) rice plants and higher concentration was observed in case of larvae as well as adult thrips. While higher concentration of *Bt* protein in case of H1C (Cry1Ac) was also observed. Normal levels of *Bt* protein were detected in case of E1 (Cry1Ab) concentration in Bt rice plants, larvae as well as adult thrips. While no *Bt* protein was detected in case of control cultivars in all tested control cultivars. No Bt protein was detected in predator *Orius sp.*

2.16. Assessment of Genetic Diversity of Pakistani Hexaploid Wheat (*Triticum aestivum* L.) Based on High Molecular Weight Glutenin Subunits (HMW-GS)

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Crop improvement program depends upon the availability of variation within germplasm that must be quantified for precise breeding. Genetic diversity estimation is important for varietal identification and proper purity maintenance. Forty eight wheat genotypes including promising lines and approved varieties have been fractionated by SDS-PAGE to determine the composition of high molecular weight glutenin subunits (HMW-GS). Four wheat varieties were used as standard checks named C-591, Blue Silver, Chinese spring and Pavon. Among all the 14 allelic variants detected d at all the *Glu-1* loci , three (Null, 1 and 2*) were found at *Glu*-A1 locus of the wheat germplasm. *Glu-B* locus was highly polymorphic. 9 subunits or subunit pairs were identified at *Glu*-B1 locus as 7+8, 7*+8, 7+8*, 7+9, 14+15, 7*+9, 13+16 and 17+18. *Glu-D1* locus consisted of 2 allelic subunit pairs i.e. 5+10 and 2+12. A salt tolerant line WSP-200 was found most diverse genotype among all experimental material. The variation detected in the HMW-GS is useful for variety identification and to understand the nature of genetic variation.

2.17. Evaluation of forensic efficiency parameters of Short tandem repeat (STR) DNA markers in Saraiki population of Pakistan

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Genetic parameters for forensic application of 15 autosomal short tandem repeat (STR) DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were evaluated from a randomly selected sample of 150 unrelated Saraiki individuals of Pakistan. The loci D8S1179, D21S11, D7S820, D3S1358, D13S317, D19S433, vWA, D18S51 and FGA were found to be highly polymorphic with observed heterozygosity > 70%, while CSF1PO, THO1 and TPOX were moderately polymorphic with observed heterozygosity > 60%. The locus D2S1338 and TPOX were found to be the most and least discriminating STR markers, respectively. Remarkably the locus THO1 was more discriminating than CSF1PO, D3S1358 and D5S818. Highest polymorphism information content (PIC) value was observed in case of D2S1338 followed by FGA, D18S51, D8S1179, vWA while it was lowest for TPOX and THO1. The loci D2S1338 showed maximum paternity index (PI) followed D8S1179, D19S433, vWA, while minimum value was observed for THO1 and TPOX. Matching probability of TPOX was more than any other loci. Thus the forensic efficiency parameters evaluated in this study would help to establish a reference database of Saraiki population for forensic DNA case work in Pakistan.

2.18. *In Silico* Study of NS5A Protein for Identification of Active Residue With Host Virus Identification

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Hepatitis C viral infection is a serious health issue worldwide caused by Hepatitis C virus (HCV). There is an urgent need of search for new direct acting antivirals due to the rapid devolvement of drug resistance. The HCV NS5A protein is involved in creating resistance against antiviral therapy and there are also many reports that vitamin A deficiency is associated with nonresponse to antiviral treatment in HCV infected patients. The present *in silico* study was designed to find out the relation between vitamin A deficiency and the NS5A protein's function in interferon resistance. Amino acid sequence of NS5A was retrieved from NCBI with accession number ABH10008. 3D structure of respective protein was predicted by online modeling programs Integrated MODELLER and I- Tasser. The best model was selected on the basis of RMSD, TM and C score. For the model evaluation, Rampage and ERRAT evaluation tools were applied. Results of these tools showed that the model was of correct topology. Since association of vitamin A deficiency with nonresponse to antiviral treatment has been proposed, so BMS411 (a vitamin A related compound) was used as a ligand for NS5A. The ligand was retrieved from ZINC database with accession number 01542874. Structure of NS5A protein, predicted using I-Tasser (Interactive Tasser), was employed for Molecular docking analysis to investigate the interaction of ligand $C_{26}H_{23}NO_3$ (vitamin A related compound)with NS5A protein. Docking analysis showed that BMS411 (a retinoid) can bind with HCV NS5A protein, the amino acid residues surrounding the ligand molecule were identified and were shown to be involved in the formation of binding pocket. Docking results also showed that this compound binds with NS5A protein like other NS5A inhibitors that bind with this protein and reduce its affinity for HCV RNA. So it can be used as potential inhibitor of this protein. The present study suggests that vitamin A or its related compounds (retinoids) could be an important modifiable factor interfering with IFN sensitivity through interaction with and inhibition of NS5A protein.

2.19. *In Silico* study of miR-132 with mRNA of Acetylcholinesterase to Investigate the Binding Affinities for Interaction

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Acetylcholinesterase (AChE) is a key enzyme in central nervous system which hydrolyzes neurotransmitter, acetylcholine (ACh) at cholinergic brain synapses. High expression of AChE in brain and muscles is responsible for diverse neurological conditions leading from neuromuscular, neurodegenerative and neuropsychological diseases. miRNA-132 is a non-coding RNA molecules which help to regulate the expression level of number of genes involved in neurological development, angiogenesis and synaptic transmission. However, miR-132 is the only miR so far that has been experimentally validated as targeting AChE, with consequences on inflammatory responses. The present study utilized tools like MODELLER, I-Tasser, ModeRNA and 3D-Dart to build a 3D model of AChE, miR-132 and mRNA. To find out the binding affinities of these macromolecules bioinformatics tools like AutoDock and Hex were used. The results as viewed by Chimera and PyMOL proved that the miR-132 binds to 3'-UTR region of AChE. The catalytic triad Ser-203, His-447 and Glu-334 were found in active site gorge of protein binds to the ligand. Thus the models of protein-ligand and mRNA-miR-132 complexes proved miR-132 as an inhibitor of Acetylcholinesterase.

2.20. ALGORITHMS AND MODELS OF ARTIFICIAL IMMUNE SYSTEM (AIS) WITH THEIR APPLICATIONS

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Due to rapid developments made in 21st Century, the field of biological sciences has also been instigated different emergent technologies e.g. artificial immune systems and artificial neural networks. There are so many characteristics in human immune system that offer successful experiments in the domain of computing systems, for instance several adaptive algorithms for fault tolerance and robustness which are collectively named as AIS (Artificial Immune Systems). This report reviews different range of algorithms and techniques investigated by many researchers in the field of AIS. At present two most widely Artificial Immune Systems generations are being practiced, where first one is based on simple theory of immune models and other is exploiting interdisciplinary relationship in order to build better perspective of immune system then finally constructing extra multipart models. Algorithms for both types have been effectively employed for different range of problems, such as pattern recognition, robotics, anomaly detection and optimization. Three major theories exist in immunological process i.e. clonal selection, immune networks and negative selection. There are a large numbers of approaches exist in the research papers which concentrate onto produce a such kind of approach which is optimized in nature, as the great work on CLONALG and other form of distributed and parallel AIRS versions. The investigation further explores a variety of algorithms and models developed so far for different types of applications.

2.21. *In-Silico* Structural and Functional Characterization of A Hypothetical Protein of Vaccinia Virus

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By the development of number of computational software's, available gene number has been increased from the last decade. *In-silico* approach also facilitates to characterize these hypothetical proteins with respect to their structure as well as function. These approaches led the annotation of vaccinia virus genome and explored many hypothetical genes. The following study is designed to estimate the effects of one of the hypothetical protein of vaccinia virus exhibiting conserved domain of *PHA02934*. This domain belongs to the *cl19668* superfamily and the homologues of this hypothetical protein reveal it a putative apoptotic inhibitor. Modelled protein showed maximum number of random coils (51.32%) with alpha helix (34.51%) and extended strands (11.06%) as secondary structure elements.

2.22 Cloning and Expression of Buffalo Prochymosin

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Chymosin, also known as rennin, is an aspartic protease which is obtained from the unweaned calf abomasum. Chymosin is formed in the form of precursor preprochymosin that converts to prochymosin and finally to its active form by autocatalytic proteolysis. The application of rennin as exogenous enzyme is in food processing for cheese manufacturing. The objective of this project was the synthesis of recombinant prochymosin of buffalo that can be used as an alternative source for animal rennet. The RNA was extracted from abomasum of milk suckling buffalo kid, cDNA was synthesized by reverse transcriptase and amplified through PCR. The cDNA of prochymosin (1.1 kb) was cloned into pCR®2.1 vector by T/A ligation and confirmed by single restriction with *EcoRI*. The prochymosin cDNA was then cloned in pET21a expression vector using *NdeI* and *EcoRI* restriction enzyme. The recombinant prochymosin was expressed in BL21 host cells using 1 mM IPTG. A band of approximately 41 kD in induced sample confirmed the recombinant prochymosin when analyzed on SDS-PAGE. The recombinant prochymosin was expressed in the form of inclusion bodies which was further subjected to solubilization and refolding. This research study is of great importance to produce recombinant buffalo chymosin that can convert the buffalo milk to cheese in high yield.

2.23 Investigation of the Neuroprotective Role of *Syzygium aromaticum* Extract on $AlCl_3$ Induced Oxidative Stress in Mice Brain

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Recent evidences suggest that aluminum is an important causal factor for increased oxidative stress in brain which is one of the triggering factor of many neurodegenerative disorders, including Alzheimer's disease. The present study has investigated the antioxidant and neuroprotective effects of *Syzygium aromaticum* extract (500 mg/kg) on an in house generated aluminum chloride ($AlCl_3$; 300 mg/kg) induced mouse model of oxidative stress. Expression of oxidative stress markers (SOD1 and Prdx6) and amyloid precursor protein (APP) was evaluated by PCR in the hippocampus and cortex specimens of mice. Differential proteome analysis of cortex and hippocampus was carried out by SDS-PAGE followed by MALDI-TOF MS/MS. An increased expression of amyloidogenic proteins APP 770 was observed in AlCl3 treated mice brain that may lead to formation of amyloid plaques. Altered expression of CREM, MLKL, TMC1, ZPI, and PRKN2 was also observedwhich are involved in various biologically important pathways. Substantial decrease in the density of Nissl substance was also observed in both $AlCl_3$ treated cortex and hippocampus.Interestingly, treatment with *S. aromaticum* normalized the changes in protein expression pattern as well as the alterations in the expression level of SOD1, Prdx6 and APP isoforms along with structural changes. In conclusion; S. *aromaticum* extract has the potential in counteracting the damage inflicted by $AlCl_3$ on brain. Further characterization of active compounds of *S. aromaticum* identified in this study will facilitate the development of more effective drugs to combat oxidative stress and its associated consequences.

POSTER PRESENTATIONS

TRACK 2

2.1-P Involvement of transcription factor (*TF*) in fiber development of Extra Long Staple (ELS)

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Global transcriptional analysis using microarray or next generation sequencing produce overwhelming amount of data and full utilization of that information is beyond the capacity of a single scientist. We made use of unutilized public microarray raw data produced by cotton fiber development studies and performed meta-analysis using bioinformatics tools in GeneSpring 13.0 -GX (Agilent Technologies). After normalization between experiments, the transcriptome of various varieties of *Gossypium hirsutum* (producing short and long fiber) and *Gossypium barbadense* (producing extra-long fiber) was compared and identified 1431 genes differentially expressed among fibers of different lengths. 574 genes showed upregulation while 844 genes were down regulated in *G. barbadense* as compared to *G. hirsutum*. In order to validate meta-analysis results, expression of 5 genes was checked in local germplasm of G. *barbadense* and *G. hirsutum* along with desi cotton by RT-real time PCR. The expression of all tested genes validated microarray data. The expression pattern of an ethylene responsive transcription factor (*TF*) and a vacuolar processing enzyme gene completely correspond to fiber lengths in cotton. TF is previously reported to be specifically expressed in cotton boll and involved in the seed oil biosynthesis. However, present study highlights that *TF* shows significantly higher expression in ELS as compared to *G. hirsutum* and *G. arboreum* during fiber development. *TF* transcription is enhanced in fiber while reduced in seeds during different stages of boll development. As *TF* shows minimal expression in desi cotton, thus introduction of this gene in desi cotton can lead to improvement in fiber quality trait.

2.2-P *SOCS-3* Gene mRNA Expression as Indicator For Non-Response and Relapse to Therapy in Pakistani Hcv Infected Patients

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Interferons (IFNs) are key cytokines in the establishment of a multifaceted antiviral response. Suppressor of Cytokine Signaling 3 gene **(**SOCS3) belongs to SOCS family and it is one of the negative regulators of cytokine signalling and IFN response that function via the JAK-STAT pathway. SOCS3 genetic polymorphism and expression influences the pathogenesis and outcome of antiviral treatment in hepatitis C virus (HCV) infected patients. This comparative study was conducted on 300 subjects divided into two major groups on the basis of therapeutic response i.e. SVR and non-responders/relapsers. ARMS PCR technique was used for SOCS3 gene polymorphisms, 8464 A/C (rs12952093), -4874 A/G (rs4969170) and -1383 A/G, (rs4969168) analysis. SOCS3 gene expression was done by using Real time PCR technique, and data was analysed using IBM SPSS 20.0. The distribution of rs4969168, rs4969170 and rs12952093 genotype frequencies between non-responders/ relapsers vs. SVR group were statistically not significant. Only the allelic frequency of rs4969170 was statistically significant ($p = <0.0001$) when patients with relapse were compared with patient with SVR /non-relapsers group. Gene expression analysis of SOCS3 showed that there was statistically significant increase of 2.27 and 3.72 folds in relative gene expression ($p = 0.004$) SVR vs. non-responders/ relapsers as compared to normal healthy control samples. Allelic frequencies of rs4969170 are more associated with the therapeutic response in relapsers vs. non-relapsers. Moreover, the gene expression analysis of SOCS3 showed a clear difference in mRNA expression of SOCS3 as an indicator of therapeutic response in our local population (Pakistan).

2.3-P Molecular characterization of begomoviruses infecting ornamental plant *Zinnia elegance*

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Begomoviruses are single stranded circular DNA viruses (belonging to family *Geminiviridae*. Highly pathogenic begomoviruses have been reported from Asia, Africa and Australia, becoming a major threat to food, fiber and ornamental plants. Recently, the ornamental plant *Zinnia elegans* was observed showing symptoms of leaf curling and vein thickening in the vicinity of Faisalabad. Leaf samples were collected from symptomatic plants to explore the possible presence of begomoviruses. Following DNA extraction, viral molecules were amplified either using rolling circle amplification (RCA) or by using universal primers in the PCR reaction. The amplified molecules by both the strategies were cloned in pTZ vector and confirmed through restriction digestion. All the cloned molecules were sequenced and analyzed for the presence of any mutation or recombination. Finally, a phylogenetic tree was constructed to determine the origin of begomoviruses infecting *Zinnia* plants. Sequence analysis showed recombination between two viruses *Papaya leaf curl virus* (PaLCV) and *Tomato leaf curl Pakistan virus* (ToLCPKV). This recombinant virus was previously un-described from plant *Zinnia elegans.* BLAST search announced close similarity (99%) of identified molecule with ToLCPKV. The results suggested *Zinnia elegans* as an alternate host of begomovirus (ToLCPKV) that can potentially provide a niche for begomoviruses circulation. Partial dimeric constructs were made by digestion of full-length molecule in two smaller fragments and cloned in pTZ (partial clones) and re ligation of full-length molecule in both of partial clones. To confirm the causative agent of leaf thickening disease in *Zinnia*, infectivity analysis will be the focus of research in near future.

2.4-P Isolation and Characterization of Genes from Sugarcane Responsive to Abiotic Stress Tolerance

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Abiotic stresses may lead to numerous physiological, biochemical and molecular changes that affect plant growth and development. Molecular approaches offer solutions to minimize such losses. A variey of stress-responsive genes including transcription factors and proteins have been identified and functionally characterized in plants. Sugarcane harbors a range of endogenous genes that directly or indirectly interact with biotic and abiotic factors. Here we report identification of an abiotic stress responsive gene from local sugarcane genotypes. Cloned gene was sequenced confirmed, analyzed using bioinformatics tools and found 99% homologous with drought responsive protein-1, predicting its role in abiotic stress tolerance. Hence, the gene could be used in screening the evolving sugarcane genotypes as well as engineering genome of other agronomic plants for abitoic stress tolerance.

2.5-P *CYP1B1* Mutations in Patients with Primary Congenital Glaucoma

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Primary congenital glaucoma is a significant cause of blindness in children. In PCG the developmental defects of the trabecular meshwork of the eye lead to the obstruction of aqueous outflow and consequent increased intraocular pressure (IOP) resulting in optic nerve damage and ultimately permanent loss of vision. The incidence of PCG varies geographically with higher occurrence in populations where consanguinity is common. *CYP1B1* is major contributing gene for PCG. The present study was aimed to investigate the mutational spectrum of the *CYP1B1* in local patients affected with PCG. The study comprised of 35 participants clinically diagnosed with PCG. DNA was extracted from blood samples of PCG patients enrolled in the study and all the coding regions of *CYP1B1* were amplified by polymerase chain reaction (PCR) using specifically designed primers. PCR products were purified and directly sequenced to find out the mutations in *CYP1B1* gene. Sequencing analysis revealed one mutation, c.1169 G>A in exon 3 of *CYP1B1* gene. This missense mutation replaces Arginine with Histidine at codon 390. This homozygous mutation was observed in 20% of the PCG patients enrolled in the study. In addition two missense sequence variants c.1294G>C (2 patients), c.1358A>G (4 patients) and a synonymous variant c.1347T>C (18 patients) were also seen in the present study. The results of the current study reflect that *CYP1B1* mutation is one of the cause of primary congenital glaucoma in Pakistani patients.

2.6-P Identification of Glucokinase Mutations in Subjects of Diabetes Mellitus having Retinopathy

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Glucokinase (ATP:D-glucose-6-phosphotransferase),depicted fully throughout hard working liver along with pancreatic islet beta cellular material, catalyzes the first task involving glycolysis along with and acts as glucose sensor and metabolic signal producer in these tissues. The enzyme plays a strategic role in glucose homeostasis and as such is an exceptional candidate for inherited imperfections predisposing to non-insulin dependent diabetes mellitus (NIDDM). The differences in allelic frequencies between individual's diabetics having Retinopathy and nondiabetic individuals were compared. After entering 30 diabetic and 30 nondiabetic subjects, found that there was the family history of diabetes (P = 0.001), which reflects the tendency of familial aggregation of diabetes. In Diabetic Patients having Retinopathy, negative 1.1-kb was more common than in normal subjects. Clinical manifestations' according to the RFLP of glucokinase gene, Random blood sugar, fasting blood sugar, cholesterol level, low density lipoprotein and high density lipoprotein level were not different between the 1.1-kb allele positive and negative group. The severity and reliability of PCR-Sequencing is validated by the fact that polymorphisms screened cover a wide genome range including both susceptible regions and or unrelated regions to diabetes. In conclusion PCR-sequencing showed qualitative differences in diabetic patients in comparison to non-diabetics. Glucokinase is the most important component in glucose detection of pancreatic islets beta cells diabetes because of glucokinase mutation can be one of the most common single gene disorders describe. It is known that a genetic variation of a human glucokinase gene, including a point mutation, causes MODY. It is confirmed through both markers sequencing as well as RFLP, concentration of plasma glucose increased and it is supposed to be the cause of diabetes, is a genetic variation in human gene including glucokinase point mutation. Diabetes is heterozygote displays in the locus, which as a mutant and a wild-type allele after a sequence of PCR products and, as well as confirmed by RFLP.

2.7-P Homozygosity Mapping of A Consanguineous Pakistani Family Affected with Oculocutaneous Albinism to Tyrosinase Gene

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The aim of this study was to screen the reported loci/ genes in the families affected with oculocutaneous albinism (OCA) and to find out the causative molecular defect segregating with the disease phenotype. It was a family based descriptive study. Ten families affected with OCA and having at least two affected individuals in their extended family were recruited for the study. Families were visited and blood samples were collected from the affected and unaffected members of enrolled families. DNA was extracted and linkage analysis was performed to screen the OCA genes/ loci. Results were statistically analyzed using LOD score program. Out of the 10 families genotyped by linkage analysis, only one family (AL03) was putatively linked to a reported gene for OCA. This family was found linked to *tyrosinase (TYR)* gene, located at chromosome 11q14.3. Statistically, the family was evaluated using the LOD score and a maximum LOD score of 1.80 was obtained with marker D11S1367. This study reports a family designated as AL03 with two members affected with OCA linked to tyrosinase gene. None of other nine families screened were found linked to the genes screened for OCA.

2.8-P PCR Based Screening for *srf*A-A ORF1 From Local *Bacillus* Species

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Cyclic lipopeptides including surfactin, iturin and fengycin families are important metabolites produced by *Bacillus* having significant role in disease control, agriculture, medicine, cosmetics, pharmaceuticals, food and petroleum industry. Some of these lipopeptides have been reported to show biosurfactant activities with surfactin being the most studied biosurfactant possessing growth inhibition activity for phytopathogenic bacteria, fungi, virus or mycoplasma. *srf*A operon of surfactin is essential for competence development and the presence of this operon of surfactin gene was confined to *Bacillus subtilis, B. amyloliquefaciens* and *B. circulans* only. In the present project, three different *Bacillus* species i.e. *Bacillus subtilis*, *Bacillus thuringiensis* and *Bacillus sphaericus* were screened for the presence of *srf*AA ORF1 of surfactin synthetase using PCR. Specific primers were designed against this ORF1 region for PCR amplification with the help of online bioinformatics tools and by using already reported GenBank data. Two isolates of *Bacillus subtilis* showed variability from the reported size of the ORF1 region (~500 bp for MBL-A and ~700 bp for MBL-B). Some nonspecific amplifications of approximately 300 bp in size were also obtained from *Bacillus thuringiensis* and *Bacillus sphaericus* and may represent some part of surfactin synthetase ORF1. Precise knowledge requires further studies of gene characterization, genetic variations and phylogeny.

2.9-P Bioinformatics Analysis of Camelysin Gene Isolated from Local *Bacillus thuringiensis*

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Bacillus strains have been widely used for the production of fibrinolytic enzymes having role in the treatment of cardiovascular disorders. Purification and overproduction of such enzymes has increased their usage in medical fields including metalloproteinases that can degrade extracellular matrix (ECM) proteins and also help in regulating the activity of other proteinases, chemokines, cytokines, growth factors and cell receptors. Camelysin, a neutral metalloproteinase converts the inactive plasminogen into active plasmin and interacts with the blood coagulation cascade proteins assisting the penetration of fibrin clots and extracellular matrix. It has been isolated from different species of bacteria like *Bacillus cereus, Bacillus thuringiensis serovar israelensis* and *Bacillus thuringiensis serovar kurstaki*. The current project was designed to isolate camelysin gene from local strain of *Bacillus thuringiensis* via PCR and the sequence obtained after sequencing was subjected to data mining by applying various bioinformatics tools and software. Dissimilarity of some nucleotides along with variance of three amino acids in the resulting protein was observed compared to reference sequence of camelysin of *Bacillus thuringiensis serovar israelensis* i.e. NQE replaced by HLK at position 182-84. The sequence attained was submitted to GenBank under accession no. KT023597. Furthermore, prediction of secondary structures, restriction enzymes, motifs structures, conserved domains and homology modelling was made accompanied by comparison of results with reference sequence. The acquired information can help in better understanding of complete structure of camelysin protein and also its function and use in clinical treatments.

2.10-P Isolation and sequence analysis of a cyclotide gene (cliotide) from local *clitoria* plant

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Plants have always been a valuable source of bioactive agents vital for our daily healthy life maintenance. Valuable agents also includes metabolites of primary and secondary importance alongwith a number other bioactive agents like proteins and peptides. Bioactive peptides of pharmaceutical importance are a potential growing field of bioactive compounds with least side effects. Cyclic peptides like Cyclotides are one of the most emerging gene encoded end to end cyclized peptides of approximately 30 amino acids. These peptides are now reported to be present in almost all plant families like fabaceae, violaceae, poaceae, rubiaceae, cucurbitaceae etc with potential of being unique in stability and drug delivery tool. Cyclotides from fabaceae plant like *Clitoria* are called *Cliotides*. The present project was therefore designed to isolate this gene from locally present *Cliotria ternatea* plants grown in the University of Agriculture Faisalabad botanical gardens. Gene amplicon was obtained from the genomic DNA template obtained by CTAB method and confirmed by gel electrophoresis. A fragment of the genetic sequence of 683 bp with a CDS sequence encoding for 135 amino acids residues was submitted in Genebank (NCBI) through BankIT (accession number KP889219). The sequence was further subjected to bioinformatic analysis and showed unique features and characteristics like having a chimera of bioactive domains of cyclotide, albumin-1, transcriptional factor NarL and glysyl t-RNA synthatases (MotifFinder). Among all these it was seen that the cyclotide domain remained the most conserved whereas few variations in sequence in rest of the reported sequence. The present findings and much more about the sequence reported can add a lot to the present understanding of the continuously increasing class of cyclotide genes and its variants for further molecular studies.

2.11-P Peptide based antimicrobial activity of *Psoralea corylifolia*

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Plants are natural antimicrobial crude drugs and have been used since antiquity for treatment of different ailments before the discovery of antimicrobial compounds. Evaluation of antimicrobial potential of medicinal plants under stress conditions paves the way to discovery of more potent antimicrobial peptides against infectious or pathogenic microorganisms. In this study, antimicrobial activity of crude extracts and different hour post induced (fungal induced) protein extracts of *Psoralea corylifolia* (*P. corylifolia)* seedlings were investigated against bacteria (*Escherichia coli, Staphylococcus aureus, Pasturella multocida* and *Bacillus subtilis*) and fungi (*Fusarium solani, Ganoderma lucidum, Alternaria alternata* and *Trichoderma harzianum*) by disc diffusion assay. Protein extracts were obtained through TCA/acetone precipitation/phenol protein extraction method. Maximum antibacterial activity was exhibited by seed crude extract against *Escherichia coli* and *Staphylococcus aureus* while maximum antifungal activity was observed against *Fusarium solani* and *Trichoderma harzianum*. Chloramphenicol and fluconazole were used as positive control for antibacterial and antifungal assay, respectively. The data were analyzed by statistical analysis through ANOVA (analysis of variance). Also decrease in activity was observed in proteinase K treated extracts which suggests that the activity was due to proteins/peptides present in the extract. SDS-PAGE analysis was performed for seed and seedling extracts with and without heat treatment and proteinase K treatment. Minimum inhibitory concentration (MIC) for bacterial as well as fungal strains was determined by 96 well plate assay method. *Staphylococcus aureus, Fusarium solani,* and *Trichoderma harzianum* possessed MIC of 0.125 µM while other test microorganisms showed minimum inhibition at 0.25 µM concentration. The strong antimicrobial potential of *P. corylifolia* against various infectious microorganisms suggests some pharmacological developments for its safe use as drug for treatment of related infectious diseases as well as resistant crop development through transgenic plants.

2.12-P Biochemical profiling of the wild *Ganoderma lucidum* isolated from *Salmalia malabarica* plant

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In the present study a wild *Ganoderma lucidum* isolated from the stem of *Salmalia malabarica* plant was analyzed for nutritional, nutraceutical, antioxidant, and antimicrobial potential. Proximate analysis showed higher contents of proteins, low fat contents, high dietary fibers and high level of carbohydrates. Extracts were prepared in different solvents, ethyl acetate, ethanol, methanol, *n*. hexane and water for the assessment of bioactivities. Antimicrobial activity was observed higher in ethyl acetate followed by ethanol, methanol, *n*. hexane and water. *Ganoderma lucidum* showed higher antimicrobial activity against Gram -ve bacteria (*E. coli* and *P. multocida*). The water extracts showed greater antioxidant potential with maximum phenolic contents (360.72±34.07 mg/g) and least IC_{50} value (45.16±5.37 mg/g). Phenolics and flavonoids contents were ranged from 60.72±12.89 to 360.72±34.07 and 45.39±8.13 to 217.5±21.32 mg/g, respectively. Water extracts showed more reducing potential which was increased with increase in concentration. Water extracts also showed higher ascorbic acid contents whereas β-carotenes and lycopene were present in minute quantity. *G. lucidum* showed negligible toxicity against human erythrocytes. The water and alkali soluble glucans were also isolated and characterized by FTIR, XRD and SEM. Comprehensive study was conducted for documentation of this wild species. So in future this species might be propagated for its commercial utilization.

2.13-P Cloning of resveraterol related Stilbene Synthase Gene from *Vitis vinifera*

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Resveratrol is a phytoalexin which is secreted by plants like grape berries and its leaves in biotic and abiotic stress. It is synthesized by the phenylalanine pathway using two basic enzymes stilbene synthase and coumarate CoA ligase and a substrate p-coumaroyl CoA. Resveratrol plays an important role in biochemical and clinical science, it is also used in pharmaceutics and cosmetics on large scale and cures many disorders like liver infections, heart troubles, skin infections, blood circulation and lipid metabolism too. It behaves as a free radical scavenger and intoxicating antioxidant to indorse the activities of numerous antioxidative enzyme. Stilbene synthase (STS) belongs to a super family of type-III polyketide synthase and a key enzyme for the biosynthesis of stilbenes including resveratrol. In this project, we focused on the isolation of the *STS* gene from *Vitis vinifera* (grape). The DNA was isolated from the plant species by CTAB method and *STS* gene was amplified using gene specific primers. The isolated gene was ligated in pTZ vector followed by transformation in *E.coli*. The cloned gene may be employed for gene expression studies, and characterization of the recombinant protein.

2.14-P Isolation and sequence analysis of defensin like genes (DEFL) from selected plants of Solanaceae family

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The spread of multi-drug resistant microbial strains and reduced range of drugs available led to an enquiry for therapeutic alternatives. Plant defensins are short peptides that show resistance against a variety of human and plant pathogens with a number of other bioactivities and characteristics. Plants besides bearing defensins also possess defensin-like peptides and genes with much more potential to be discovered as a new class of peptides. Antimicrobial defensin-like peptides have also been isolated from Solanaceae plants. We therefore focused our research towards screening/isolation of defensin-like genes from selected plants of solanaceae family like *Solanum tuberosum, Solanum nigrum, Solanum lycopersicum, Withania somnifera, Datura stromonium.* Plant's genomic DNA was isolated by CTAB extraction method. The presence of DEFL gene was confirmed by PCR by specifically designed primers in all plants except *Solanum nigrum*. However out of all screened ones, a novel unreported gene sequence of DEFL of 472 bp was isolated, sequenced and submitted in GenBank (NCBI) with accession number KT371458. Future expression and bioactive studies can further help us exploring its unique bioactive role and the present study also helps in screening defensin-like peptides in plant families.

2.15-P Diversity and Relative Abundance of Order Araneae, Hymnoptera and Pulmonata in Cereal Crops of District Faisalabad

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Diversity and relative abundance of Arthropods vary according to the nature of crop as well as time and space. However, ecological factors abruptly influence their existence. Whereas, cereal crops such as rice, wheat and maize are staple food for mankind; and are important habitat for millions of insect taxa. Insects also play many roles for better outcomes of these crops. So, present study was designed to accord the diversity of order Araneae, Hymenoptera and Pulmonata around the Faisalabad city. From the overall results, total 1948 specimens were collected and identified. From the total, 655 specimens pertaining to order Araneae, 633 specimens for order Hymnoptera, while 660 specimens for order Pulmonata were collected from three crops. For order Araneae, maximum relative abundance 50.39% ($n \geq 130$), 60.33% ($n \geq 146$) and 34.19% ($n \geq 53$) was recorded for genus *Oxyopes* (Family Oxyopidae) in Wheat, Rice and Maize fields respectively. The present insect data base will be helpful in designing future estimate of diversity of these orders.

2.16-P Molecular Analysis of GALT Gene in Patients with Galactosemia

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Type I galactosemia is an inborn error resulting from mutations on both alleles of *GALT* gene which leads to absence or deficiency of galactose -1-phosphate uridyltransferase (GALT), the second of three enzymes catalyzing the conversion of galactose into glucose. Type I galactosemia is further classified into severe "Classical" and mild "Duarte" galactosemia. Classical galactosemia is frequently associated with Q188R, S135L and K285N mutations and N314D is associated with Duarte galactosemia and is wide spread among various worldwide populations. The objectives of this study are to identify most common mutations Q188R, S135L, K285N and N314D for patients with classical and Duarte galactosemia. To correlate genotype with its phenotype.The present study aims at detecting Q188R, S135L, K285N mutations and N314D variant in the *GALT* gene by using amplification refractory mutation system (ARMS). ARMS assays were established using standard DNA samples and were used for eight galactosemia patients and 190 unrelated normal subjects all of Pakistani origin. S135L and K285N mutations were present neither in galactosemia patients nor in normal subjects. Only one galactosemia patient carried Q188R mutation that was in homozygous state. However, N314D variant was frequently found in affected 7out of 16 alleles and normal subjects (55 out of 380 alleles). This finding indicates that Duarte allele D314 might be far common in Pakistani population than in European and North American ones.

2.17-P PCR based identification and phylogenetic data analysis of *Streptococcus equisimilis subspecies equisimilis* (SESE) strains isolated from horse nasal fluid

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The taxonomic status and structure of *Streptococcus equisimilis subspecies equisimilis* (SESE) and *Streptococcus dysgalactiae subspecies equisimilis* (SDSE) have been the object of much confusion. Bacteria belongings to this subspecies level are usually referred to as Lancefield group C in clinical stings. The aim of present study to develop an improved basis for identification and phylogenetic analysis of individual species at subspecies level of SESE and SDSE. We critically investigated the morphological and genetic relationship of SESE and SDES, which may share the Lancefield group antigens, and 16sRNA gene sequence combined with biochemical characterization. Phenotypic and biochemical characterization created a compact difference between both taxa at subspecies level further confirmation was done by using universal primer of 16sRNA gene. Both species at subspecies level shown a close relationship which indicated their common ancestory within genus Streptococcus, while phylogenetic analysis also confirmed the divergence of SESE from SDSE of the pyogenic group *Streptococci.* The results provide an improved basis for the identification of clinically important species and explain the overlapping spectrum of species associated their natural habitat.

2.18-P Cloning of RNA interference related genes from *Nicotiana benthamiana*

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RNA interference (RNAi) plays an essential role in eukaryotic system development, gene regulation, antiviral defense and heterochromatin formation. Important proteins like Argonaut, Dicer, RNA-dependent RNA polymerase (RDRPs) and double stranded RNA binding proteins (DRBPs) like DRB1, DRB2a, DRB2b etc. are involved in different steps of RNA silencing. These multifunction proteins are involved directly or indirectly in miRNA and siRNA production. Keeping in view their importance in RNAi, this study was designed to clone these genes. Firstly, the genes involved in RNAi in *Nicotiana benthamiana* (a model plant) were identified and their sequences were obtained from public database. Total RNA of *N.benthamiana* plants was extracted through phenol-chloroform method. RNA quality was checked by running on 1% agarose gel. Gene specific primers were designed and the genes were amplified through RT-PCR or PCR from genomic DNA. These genes were cloned in TOPO 2.1 vector or pTZ57R/T cloning vector. The cloned genes include three important members of Argonaut protein family i.e. *AGO1b, AGO4a, AGO5,* four members of DRB protein family i.e. *DRB1, DRB2a, DRB2b, DRb3* and one member of DNA-dependent RNA methylase protein family i.e. *DRM3*. These constructs provide a useful resource of these proteins to study viral interaction with RNAi machinery.

2.19-P Controlling Cotton Leaf Curl Viruses with Novel Talen Approach

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Cotton leaf curl virus disease (CLCD) has proved as the biggest malady to the cotton crop in last couple of decades. To date different technologies have been to suppress cotton leaf curl virus (CLCV) like conventional breeding, antisense technology, RNAi etc. TALENs (Transcription Activator-Like Effector Nucleases) offer a swifter and more exact route for suppression of virus molecules. The main objective of this study was to suppress cotton leaf curl viruses using TALEN technology. The sequences of most devastating CLCD causing viruses in Pakistan were retrieved from NCBI and were subjected to Multi Sequence Analysis CLUSTALW to target a consensus sequence for broad-spectrum inhibition of viral replication in plants. The consensus sequence was further analyzed for potential TALEN target sites using TALEN™ Hit software. TALEN was constructed against target sequence of *Rep* gene of CLCV using Golden Gate and fastTALEN strategy. TALEN efficiency for virus suppression was assessed in tobacco through transient assay. Presently, we are working on stable transformation of cotton for disease resistance assay to observe virus suppression in cotton. Results of transient assay prove that TALENs could represent a reliable and robust method of genome targeting for virus resistance.

2.20-P Evaluation of Anticancer Effects of *Cassia fistula* Seed Extracts on HeLa cell Line

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When normal homeostasis i.e. the equilibrium between apoptosis and proliferation is disturbed, it cause stimulation of abnormal cell proliferation that lead to malignant activities. Apoptosis is a vital process in the human body as it facilitates to maintain homeostasis. Mainly, cancer happens due to imbalance in proliferation and apoptosis. Stem cells (SCs) are basically unspecialized cells and they are potentially able to differentiate into specialized cells. The umbilical cord (UC) was discovered as the first fetal tissue in which stem cells are found. UC-MSCs can be used as regenerative drug and different anticancer therapies. Our study aims to evaluate the effect of seed (extract of Ethanol, Acetyl acetate and petroleum ether) of *Cassia fistula* (Cf) on both UC-MSCs and HeLa cells. UC-MSCs were used as controlled group. Ability of Angiogenesis was checked via ELISA of VEGF. ELISA of Annexin-V and p53 and LDH assay was performed for the estimation of apoptosis in HeLa cells and UC-MSCs. For the estimation of anti-proliferation, cell viability and cell death in HeLa cells and UC-MSCs, MTT assay, trypan blue and crystal violet was done. Antioxidant enzymes activity (SOD, GSH, CAT and APOX) was also observed. HeLa cells showed reduced angiogenesis, low level of proliferation, high apoptotic level, reduced cell viability, increased cell death in HeLa cells. Whereas anti-oxidative enzymes showed the low levels in treated cancer cells as compared to normal ones. It is found that the seed of *C. fistula* with HeLa cells induces apoptosis and oxidative stress along with inhibition of proliferation and angiogenesis may strongly have profound effects on growth suppression of HeLa cell lines.

2.21-P Evaluation of population genetic data of 15 Short tandem repeat DNA markers in Pakistan for the establishment of DNA database

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Allele frequencies and various statistical parameters of 15 autosomal short tandem repeat (STR) DNA markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) were evaluated from a sample of 661 unrelated individuals, living in Khyber Pakhtoonkhwa, Southern and Central Punjab province of Pakistan. D8S1179, D21S11, D7S820, CSF1PO, D3S1358, D13S317, D16S539, D19S433, vWA, D18S51 and FGA were found to be highly polymorphic with observed Heterozygosity > 70%. THO1 and TPOX were least polymorphic with observed Heterozygosity 50 and 34.2% respectively. The investigation of statistical parameters revealed that D2S1338 showed highest power of discrimination, power of exclusion, typical paternity index, and polymorphism information content followed by D18S51, FGA, D8S1179 and vWA. Maximum matching probability was observed in case of THO1, while D2S1338 showed minimum matching probability. The combined random match probability of these STR markers was found to be 3.1×10^{-21} in Pakistani population. Therefore, evaluated allelic frequency data and parameters for forensic application would not only be useful for identification of individuals but also lead as a foundation for the establishment of STR database in Pakistan.

2.22-P Relationship Testing using AmpFlSTR Identifiler Plus in Pakistani Population

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Buccal swab samples from 125 related and unrelated individuals were subjected to DNA extraction and amplification using AmpFlSTR identifiler plus kit that were subsequently genotyped using capillary electrophoresis. 15 STR markers D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA along with additional amelogenin for gender discrimination were analyzed in this study. Allelic frequency distribution and degree of homozygosity or heterozygosity were calculated for the aforementioned 15 loci in the sample population, using gene counting method.

Paternity index, combined paternity index and probability of paternity were calculated by applying statistical tools for the 62 trios tested in this study. The values of Max CPI, Min CPI and Probability of Paternity were observed to be 3014935823, 94498 and 0.9999999425599 respectively to set the ranges. Although single step mutations were observed in few individuals, however parentage was significantly established in all the families tested in this study. Statistical sibship analysis was performed on the same 62 trios which were subjected to parentage analysis. 80% of the cases showed LH of FS > LH of HS > LH of UR. The max and min values of likelihood ratios for FS vs UR were calculated for both true sibs and unrelated individuals to determine the ranges. In approximately 90% of the trios in which parentage was significantly established, offspring could not be excluded as being the biological brother or sister of each other.

2.23-P Evaluation of DNA yield obtained from Copan Flock swabs and comparison with polyester tipped applicators for forensic DNA analysis in Pakistan

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This study was designed to evaluate Copan Flock swabs for DNA yield and comparison of DNA yield obtained with polyester tipped applicators (Fisher Brand), used for forensic casework analysis at Punjab Forensic Science Agency, Lahore. Buccal cells were collected by swabbing inside cheek. Pre wet swabs were used for swabbing of finger nail scrapping. Swabs were taken from the mouth of a plastic water bottle. Extracted human DNA was quantified by Plexor® HY human/human male DNA quantification kit as described by manufacturer protocol (Promega Carporation Madison, USA). AmpF*l*STR® Identifiler® Plus PCR Amplification Kit was used for the simultaneous amplification of 15 autosomal STR loci and a gender determination marker (Life Technologies Foster City, CA, USA). Detection and separation of amplicons was carried out by capillary electrophoresis on ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Mean DNA yield of Copan Flock swab taken from buccal swab, nail scrapping and swabbing from bottle was 12, 36, and 2% higher than cotton tipped applicators used to swab. Thus the overall yield of DNA obtained from Copan Flock swabs was found to be higher than cotton tipped applicator (Fisher Brand USA)

2.24-P *In silico* Analysis of *NRT2* Gene Family in different plant species

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For Higher Plants, N sources are mainly composed of Nitrate (NO_3^-) and it is principally acquired from soil through NO_3^- transporters (NRT). There are three sub-families of NRT transporters in plants as NRT1, NRT2 and NRT3 containing 53, 7 and 2 members in *Arabidopsis thaliana* respectively. Current study is focused on *NRT2* High affinity nitrate transporter gene family in chickpea (*Cicer arietinum*) and includes *in silico* identification and characterization of *NRT2* genes in newly sequenced *Cicer arietinum* genome. In this study a comprehensive genome wide analysis of *NRT2* genes/proteins is performed which comprise of promoter analysis, phylogenetic analysis, and conserved domains analysis. Moreover, comparative analysis for *NRT2* genes of six plant species (mono- and dicots) have been performed. This study will be a step forward to predict the functional characterization of *NRT2* genes in chickpea.

2.25-P Association Mapping of Grain Quality Traits on Chromosome 1B oF Wheat (*Triticum aestivum* L.)

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The present study was designed considering the importance of grain quality traits, genetic diversity and marker-trait association analysis in wheat. A significant amount of genetic diversity was found for various seed traits though the genotypes included in the study were found structured. The extent of polymorphism was high with a range of 2–13 alleles and average of 6.5 alleles per locus. Population structure was detected with 30 unlinked SSRs that divided the population of 92 genotypes in three sub-populations. Extensive LD extent was found on chromosome 1B with 42 SSRs specific for 1B chromosome. Marker-trait associations were determined using mixed linear model, where, population structure and kinship calculated on the basis of unlinked markers were covariated with 1B specific markers and traits data. Eight QTLs for five traits including protein, gluten contents, test weight bread and chapati making quality. Protein content, test weight, bread quality and Glu-B1 were found significantly associated with primers WMC419 (32 cM); WMC128 (30 cM), WMC419 (32 cM); WMC818 (17 cM) and WMC416 (44 cM), respectively.

2.26-P Use of Next Generation Sequencing (NGS) for SNP Identification in Salt Tolerant Rice Genotypes

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High level of soil salinity is one of the major abiotic stresses that limit rice production all over the world. Different strategies are adopted to overcome this difficulty i.e. breeding and selection of salt tolerant rice genotypes. Six rice genotypes classified as tolerant (FL478, SAL-9, SAL-10 and SAL-11) and moderate tolerant (SAL-12), and susceptible (IR64) against salt stress were used in this study for identification of SNPs in salt tolerant candidate genes/QTLs. High throughput sequencing of these 6 genotypes was carried out using double digested Restricted Associated DNA (ddRAD) approach. We obtained a total of approximately 7 million clean reads covering 1,563 MB of sequence data, with an average of 1.16 million clean reads per sample and the mean read length of 93 bp. Preliminary data analysis revealed that on average, 95% of the total bases sequenced had a quality score of at least 20. The clean reads were then aligned with available Nipponbare rice reference genome in database for the identification of SNPs between different genotypes and as compared to the reference genome. We detected a total of 202,875 raw variants with an average of 33,812 SNPs per samples. These raw variants were filtered with an average of 24 X coverage and identified 73,189 SNPs and 5,630 InDels all over the genome in all 6 genotypes used. Maximum number of SNPs and InDels were observed in salt tolerant candidate genes/QTLs. Details of identified variation in different genomic regions and their affect on salt tolerance in rice will be discussed. This GBS analysis of rice genotypes would help us to understand the genetic basis of salt tolerance in rice, which is a complex trait involving multiple factors.

2.27-P Molecular Genetic Analysis of Inborn Errors of Metabolism Causing Intellectual Disability

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Inborn errors of metabolism (IEM) form a heterogeneous group of rare inherited disorders in which the synthesis, metabolism, transport and/or storage of metabolites or molecules is disturbed. Some of the IEM may cause intellectual disability. In various population across the globe so far more than 500 IEM have been reported. Fortunately, about 89 such disorders causing intellectual disability are treatable if diagnosed at an early stage. Few of such intellectual disability disorders could be identified by genetic analysis of respective genes. Diseases causing changes in Creatine (Arginine: glycine amidinotransferase deficiency, *GATM*), Vitamins/co-factors (Biotin responsive basal ganglia disease, *SLC19A3*), Thiamine-responsive encephalopathy, *SLC19A3*)., Cerebral glucose transporter deficiency (*SLC6A19*), Mitochondria (Co-enzyme Q10 deficiency, *COQ2*; MELAS, *MTTL1*), Urea cycle (l.o. NAGS deficiency, *NAGS*), l.o. CPS deficiency, *CPS;* Creatine transporter deficiency, *SLC6A8*, Glucose transport and regulation (Hyperinsulinism-hyperammonia syndrome, *GDH*), Lysosomal (Niemann-Pick disease type C (*NPC1* & *NPC2*), Amino acids (Serine biosynthesis defects, *PHGDH, PSAT, PSPH*), Sjogren-Larssen disease, (*FALDH*) can be diagnosed by identifying the respective mutations.

2.28-P Role of microRNA in Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) is the most common leukemia throughout the world representing about 80% of cases of acute leukemia in children. Current diagnostic procedures including bone marrow biopsy for diagnosis of ALL are invasive, unpleasant, and inconvenient. So the time is to get certain diagnostic markers that either replace the invasive procedures or minimize their utility by supporting clinicians to provide a clear picture for the diagnosis of ALL. A sensitive, specific and cost effective microRNA profile may be a good alternative in under developed countries. In the present study, a panel of microRNAs with their increased and/or decreased expression in ALL patients was quantified by real time PCR. Mir-15a and Mir-10b showed down regulation in ALL samples as compared to normal samples (control). The altered expression profiles of microRNAs in ALL patients may provide the basis to utilize microRNA signatures as a novel biomarker for early diagnosis, prognosis and treatment of ALL. Hence, in this study, we aim to collect blood samples from normal and intellectually disabled children, which will be further analyzed for genetic (PCR based i.e. PCR-RFLP, ARM-PCR and RT-PCR/TaqMan) assays to identify such disorders. Once such assays are established, this will help in the early diagnosis of kids at an early stage to avoid intellectual disability.

2.29-P Isolation and cloning of *GDF-11* gene from *Oryctolagus* Species

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A family of super-protein is Transforming Growth Factor- β (TGF- β), which contains TGF- βs, activins, inhibins, Growth Differentiation Factors (GDFs), Glial Derived Neurotrophic Factors (GDNFs), Nodal, Lefty, and anti-Mullerian hormone. GDF-11 is one of the members of TGF-β family which involves various type of metabolic functions regarding muscle regeneration and recovery from age-related diseases like cardiac hypertrophy and neuronal disorders. This project was focused on isolation and cloning of GDF-11 from *Oryctolagus* species (Rabbit). The rabbit nucleotide sequence similarity with human nucleotide is 86% and protein similarity is 99%. DNA was isolated from blood of rabbit through DNA isolation kit and confirmed on agarose gel electrophoresis. The DNA was amplified by a) Phusion blood direct PCR b) Normal PCR c) Touch down PCR. *GDF-11* gene was amplified by specific primers sets, the size of amplified gene was 1200 bp. The gene was purified by gel extraction kit. The purified sample was sequenced to check the sequence of *GDF-11* gene. The gene was cloned in pTZ vector followed by transformation in *E. coli*.

2.30-P Genetic Mapping in Heredity Retinal Disorders

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Hereditary retinal disorders are heterogeneous group of disorders. With recent advances in human genome mapping, including linkage analysis and study of genetic markers with disease phenotype have increased the number of causative genes. The objective of the study is to find disease causing genes through linkage analysis in two Pakistani families (A, B) with retinitis pigmentosa. Family A and B affected with retinitis pigmentosa, failed to establish linkage in any of the previous known gene/loci such as *BBS8* and *ZNF513* by selected markers in Pakistani population. Genome wide search for the identification of causative gene is suggested for future studies.

2.31-P *In silico* Analysis of Ethylene Response Factors (ERFs) in maize (*Zea mays* L.*)*

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Transcription factors are very important for gene regulation in many plant species. Among various families of transcription factors, the members of AP2/ERF transcription factors family are well known for their roles in regulating many stress responses in plants. The AP2/ERF superfamily is defined by the AP2/ERF domain, which consists of about 60 to 70 amino acids and is involved in DNA binding. ERF transcription factors are a huge class of DNA-binding proteins, the domain of which binds with a specific sequence known as GCC box found in promoters of genes. In this study we performed a comprehensive genome wide analysis of 105 ERF family in maize using several computational techniques. We performed phylogenetic analysis,conserved motif analysis , chromosomal localization, gene structure analysis, multiple sequence alignment of ERF domain for these ERF genes. The phylogenetic analysis led to classification of these *ERF* family members into 10 major groups and subgroups within the groups and showed evolutionary relationship among these groups on the base of various protein motifs and intron/exon structure of the genes. The mapping of different ERF genes on 10 maize chromosomes was done which revealed that all the chromosomes contained ERF factors. Chromosome1 had the most of them (17) while chromosome3 contained the least (8). Interestingly most of the genes have no exon and some of them have one or two exon. Moreover, 3D structures of few ERF proteins have been developed using Phyre 2 tool. The results of the present study provide important genomic information for the stress responsive ERF transcription factors in *Zea mays*.

2.32-P Genetic and Biochemical Analysis of Biological Risk Factors Causing Cardiovascular Problems in Type 2 Diabetic Patients

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Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, and/or reduced secretion/action of insulin, which leads to several grave complications including cardiovascular problems. The major risk factors for diabetes are genetic susceptibility, physical inactivity, hypertension, obesity, fat/carbohydrate rich diet. About 10% of Pakistani population have diabetes, and 90% of all diabetics have Type 2 DM (T2D), which is a major cause of deaths owing to cardiovascular diseases (CVD) as it increases the risk of CVD related death by 2-4 times. The main reasons for CVD is the elevated level lipids (e.g Triglycerides, Cholesterol, LDLs, VLDLs) and free fatty acids (FFA) and polymorphism in susceptible genes. To prevent CVD in T2D patients, it is necessary to properly manage the circulating FFA according to health needs by increasing physical activity and diet management. Fatty acid profiling in this regard is of vital importance, which will be done in this project by extraction of circulating FFA, their derivatization to fatty acid methyl esters (FAME) and then analyzing them through GC-MS. Polymorphic analysis of susceptible candidate genes/variants will also be done by genetic assays (e.g. PCR, RFLP, TaqMan etc) to find variations which are associated with T2D patients having CVD complications. From this study, we hope to develop and validate GC-MS based method for fatty acid profiling along with identification of fatty acid and DNA polymorphism based biomarkers to assess CVD risk in T2D patients.

2.33-P Overview of extended spectrum beta-lactamases carrying Escherichia coli in wild migratory birds of Pakistan

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Extended spectrum beta lactamases (ESBLs) are plasmid mediated enzymes, responsible for resistance in bacteria to 3rd generation cephalosporins.With focus on environmental dissemination of antibiotic resistance, the proposed study investigated prevalence of ESBLs *E. coli* in wild migratory birds in Pakistan. 100 migratory bird fecal samples were screened for the detection of ESBL *E. coli*. Specimens were collected from diverse wetland areas of Pakistan during September 2013-March 2014. Phenotypic confirmation was done with (Remel RAPid) Kit. Genotypic confirmation of *E. coli* was done by PCR for uid gene. Pure *E .coli* isolates were investigated for antibiotic resistance against ceftazidime (CAZ-30), cefotaxime (CTX-30) and ceftriaxone (CRO-30) according to EUCAST. Double disc synergy tests confirmed ESBL bacteria.For molecular characterization of ESBL *E. coli*, PCR was conducted to amplify SHV,TEM, & CTX-M genes. Focusing on CTX-M, further grouping into CTX-M9, CTX-M1 & CTX-M2 was done with PCR. Phylogenetic characterization of *E. coli* isolates used Multiplex PCR, targeting chuA, yjaA & TspE 4 genes to categorize the strains in class A, B1, B2 and D. Antibiotic susceptibility with some other class of drugs was checked according to the EUCAST criteria. 40/100 fecal isolates carried ESBL *E.coli.* On molecular characterization, 26 isolates carried *bla*-CTX-M, while 19/26 co-carried *bla*-TEM. On further molecular division, 21/26 were CTX-M-15 followed by 4 carried CTX-M-1 & 1 carried CTX-M-3. Phylogenetic classification revealed 17/26 isolates belong to class A of *E. coli* while 4,3 & 2 belonged B1, B2 and D respectively. Resistance among other groups was; Chloremphenicol 92%,Gentamycin 85%, Tetracyclin 82%, Meropinum 39%, Nalidixic acid 27%, Tigeglycine 16% & Sulfmethoxazole/trimethoprim 12%. No isolate was resistant towards imipenum.

2.34-P *In Silico* Characterization of Petroleum Replica Producing Genes

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If any country is self-sufficient in energy then excel in its economy is guaranteed. According to an international analysis, the oil and gas reserves will be finished into the second half of current century. For that reason, to attain self-sufficiency, alternative energy resources are being explored. Biofuels are attractive alternative energy resources because of its environmental benefits and the fact that it is made from renewable resources. The cost is the main hurdle in commercialization of the product. At present the biofuels available require extensive downstream processing and are not well-suited with the latest combustion engines. To overcome this problem it is essential to generate fossil fuel replacements. The ideal biofuels therefore should be chemically and structurally identical to the fossil fuels. Our study is based on a previous study in which the activity of the fatty acid (FA) reductase complex from Photorhabdusluminescens was coupled with aldehyde decarbonylase from Nostoc punctiforme to use free fatty acids as substrates for alkane biosynthesis. Their results revealed that artificial metabolic pathways can be designed and implemented for the production of renewable, industrially applicable fuel molecules. Fatty acid reductase complex is encoded by the genes luxC, luxE and luxD. These were isolated from *Photorhabdusluminescens*. Aldehyde decarbonylase was isolated from *Nostocpuniforme* In the current study, alternative sources for FA reductase complex and aldehyde decarbonylase were found. We have used protein alignments of these genes from different sources with an aim to identify petroleum replica producing genes. This approach not only provides alternative sources from which these genes can be isolated but also how closely these genes are related to already tested petroleum replica producing genes.

2.35-P Isolation and Sequence Analysis of Defensin-Like Gene from *Medicago Sativa*

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The advent of targeted antimicrobial drugs over the past few decades has invigorated but problems sustain to arise with respect to resistance and low specificity of currently available drugs. Plants antimicrobial peptides (AMPs) as potential natural molecules represent a hit towards the discovery of a novel generation of drugs against pathogens. Defensins express their importance in large quantities in pharmaceutical or industrial applications. A single gene provides natural resistance towards numerous pathogens. In plants several additional classes of cysteine-rich peptides have been discovered such as defensin-like (DEFL) polypeptides. The identification of such a broad family at the same time offers new chances for the production of novel antibiotics and food crops development. In the present work, studies have been focused on the isolation of defensin-like genes from *Medicago sativa* plant of *fabaceae* family using computational analysis for predicting novel antimicrobial genes. The presence of DEFL gene was confirmed by PCR which further sequenced and analyzed. A novel unreported gene of DEFL was discovered, sequenced and submitted in GenBank (NCBI). The sequence analyzed through gene BLASTn and phylogentically found to be closed to the defensin gene of *Cicer arietinum*. However it was found that this is for the first time that a defensin-like gene encoded for the peptides with properties including antimicrobial, anti-cancerous and antioxidant were reported from *M. sativa.* The present project can add new defensin-like peptides in the already present bioactive peptides class with hidden bioactive roles that can be explored further for its molecular expression studies.

2.36-P Gene Stacking in Plants for Virus Resistance using Site Specific Recombinases

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Losses due to viral diseases are posing a serious threat to crop production. Quick breakdown of resistance to viruses like CLCuV demands application of a proficient technology to engineer durable resistance. Gene stacking has recently emerged as a potential approach for integrating multiple genes in crop plants. In present study, we used recombinase technology for site specific gene stacking. pG-Rec plasmid was constructed for recombination by inserting Rec cassette in pGreen-0029 plasmid. This Rec cassette was excised from pJet-Rec plasmid. Resulting plasmid was of 4.8Kb with npt-II as an antibiotic resistance gene. For introducing resistance to leaf curl disease in cotton, transformation protocol through particle bombardment has been established by inserting PG-Rec plasmid in cotton varieties Z-33 and Z-231. Embryos were excised from germinating seeds and shot with particles coated with PG-Rec. After transformation, embryos were selected on selective media to generate whole plant and finally develop a target line for gene stacking. Plants were transferred to soil and PCR was performed to confirm transformation. The developed target line will be used for further transformation of genes through site specific gene integration by using *Bxb1* and *Cre* recombinases. In the next step we are stepwise stacking transcription activator like effector nucleases (TALENs), (CRISR/Cas9) and guided RNAs (for Rep gene of CLCuV) involving addition of the gene of interest and removal of marker gene to recycle it with the next gene for stacking. Consequently, transgenic marker free plants will be produced with one or two genes stacked at the specific site. These transgenic plants will be tested for resistance against various strains of cotton leaf curl virus (CLCuV).

2.37-P Genetic monitoring of *Wallago attu* in Jhelum river by molecular markers

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Five microsatellites were used to study the genetic diversity and genetic structure of six wild Populations of *W. attu* in Pakistan (the Jhelum River). At least 30 fish samples were collected from six different sites of River Jhelum, Punjab. Data was analyzed by using different softwares including FSTAT and TFPGA. A total of 23 alleles were found in 177 fish samples. All microsatellite loci were found to be polymorphic whose number of alleles changed between 3 (WAM-8, WAM-17) and 9 (WAM-28). The mean numbers of alleles per population were between 3.8 and 4.6. The F_{ST} value 0.0242 showed little genetic differentiation among over all loci. The values of average observed heterozygosity were ranged from 0.3450 to 0.4400 for all of six populations whereas average expected heterozygosity ranged from 0.5363 to 0.5777. The deviation from *HWE was* significant. The results showed genetic decline in this valuable fish resource. The findings of the purposed study would have great worth for management, conservation as well as least kinship selective breeding programs of *W. attu*.

2.38-P Bio-efficacy of Tea (*Camellia sinensis*) leaves blend with Cinnamon (*Cinnamomum cassia*)

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Pakistan being an agricultural country, enjoying a variety of crops required for ever increasing population. Pakistan is an exporter of much cash crops while on the other hand investing substantial on importing Tea (120 k Mt amounting Rs.40 billion) to encounter the demand for growing population (3.26 % annually). Tea beverages are known throughout the world because for their health benefits and stress relieving properties. Tea blending is the creative experience of mixing more than one type of tea and or addition of valuable spice for a unique taste, aroma or health benefits. Cinnamon blends with tea extracts (Green or Black) turn to be an effective chemo-preventive agent for antimutagenic, antidiabetic, antibacterial, anti-inflammatory, and hypocholesterolemic qualities. The presences of precious aromatic compounds like Dodecene (0.28%), Cinnamaldehyde (79.75%), Coumarin (2.01%), Methoxycinnamaldehyde (10.74%), Caffeine (81.68%), Hexadecanoic acid (5.68%), Octadecenol (0.43%) and Eicosanoic acid (0.96%) by GC-MS analysis contributed flavor to tea after blending. Black tea and Green tea leaves are rich in bioactive phyto-constituents while tea blends with Cinnamon makes it more powerful drink for hypertensive and diabetic subjects due to the presence of vital nutrients. Antioxidant and antimicrobial activity of tea blends have also revealed affirmative outcomes as tea polyphenols and trace minerals showing essential to human health. Tea blending with promising cinnamon spice is recommended due to its synergistic effect to consumers.

TRACK 3

INDUSTRIAL/ENZYME BIOTECHNOLGY, BIOCOMPOSITES AND BIOMATERIALS

TRACK COMMITTEE

1. **PROF. DR. MUHAMMAD ASGHER (FOCAL PERSON)**
2. **DR. MUHAMMAD ANJUM ZIA**
3. **DR. MUHAMMAD JAVED ASAD**

KEYNOTE LECTURES

TRACK 3

K-3.1 Bio-based Products and Materials from Renewable Resources

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The shift from petroleum based polymeric products and materials to bio-based products/materials is rapidly accelerating. The Polymeric materials are important commercial materials with applications in very different areas such as automotive, aerospace, construction, electronics, biomedical and other industrial fields. In the recent years, the development of the nanostructured materials has further broadened their application in many other fields. However, the depletion of fossil fuel resources, the lack of space for landfills, concerns over emissions during incineration, and environmental pollution has spurred efforts to develop high performance materials which are eco-friendly and biodegradable. Therefore, more recently, the research efforts have been directed towards the development of bio-based materials and nanomaterials to supplement and eventually substitute the existing petroleum-derived counterparts. These bio-based materials are of particular interest because of being environmentally friendly, degradable and sustainable. These emerging biomaterials can be the future high performance materials for several industrial applications. In this lecture, I will present some recent examples of bio-based materials and nanomaterials from renewable resources developed by our group at University of Alberta.

K-3.2 Investigation of Biological Process for the Conversion of Bark Biomass to Bio-based Polyphenols

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Due to increasing waste production and disposal problems arising from synthetic polymer production, there is a critical need to substitute these materials with biodegradable and renewable resources. The concept of green polymers has become more appealing due to the presence of large volumes of processing residuals from the timber and pulp industries. This, in turn, supports the idea of developing new polymers based on bark extractives. In this study, three comparative treatments i.e., enzymatic, alkaline, and UV/H_2O_2, have been conducted for the extraction of beetle infested lodgepole pine (BILP) and mixed aspen barks polyphenolic extractives. Use of laccases as biocatalysts to affect and enhance the catalytic properties of enzymes has been shown to be a promising solution for bark depolymerization. Furthermore, laccases are suitable for biotechnological applications that transform bark biomass into high valued bark biochemicals. Bark depolymerization was conducted in submerged fermentation (SF) and polyphenols/polyaromatic compounds were identified after three weeks when production media (PM) was induced with 50 mg/100 mL of each type of bark during the lag-phase. During SF where honey was used as a natural mediator substitute (NMS) in the PM, laccase activities were about 1.5 times higher than those found in comparable cultures without honey in the PM. These samples were analyzed by GC-MS. The laccase enzyme was purified using UNO® sphere Q-1 anion exchange chromatography and the molecular weight was determined to be ~50kDa on 10% SDS-PAGE and laccase kinetic parameters including maximal velocity (V_{max}), Michaelis constant (K_m), and turnover number (K_{cat}) were calculated from Lineweaver Burk plot. Py-GC-MS analysis of bark showed differing effects of fungal activity on bark composition. Polyphenolics were separated in reverse-phase mode using HPLC at two selected wavelengths of 290 and 340 nm to improve separation.

K-3.3 Production, and characterization of cellulases from a Thermophilc *Bacillus* strain 60-4

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Thermophilic strains were isolated from garden soil. Among them, one strain was tentatively identified as *Bacillus* strain 60-4. It produced maximum extracellular endoglucanase (57 IU/L.h), exo-glucanse (48 IU/L.h) and β-glucosoidase (75 IU/L.h) on 0.5% cellobiose after 24 h fermentation at 55^{o}C. The defibrillation activity of cellulases was determined on cotton wool as the amount of microfibrils removed from cotton wool in the presence of cellulases in 40 ml of 2 mM Tris-HCl (pH 7.5), then the reaction mixture was incubated at 60 °C for 60 min in shaking water bath at 60 rpm. The defibrillation activity was calculated as the number of OD units per mg of enzyme. Enzyme showing an OD 610 of 0.3 was considered as one unit of defibrillation activity. In general, detergents contain non-ionic and anionic surfactants and bleaching agents (oxidizing agents) that denature enzymes. Therefore, endo-glucanases for laundry applications should possess ability to withstand these agents in the reaction mixture as well as have defibrillation activity. The concentration of SDS, or sodium hypochlorite (oxidizing agent) that inhibited 50% (I_{50}) of the CMCase activity of endoglucanse without SDS or sodium hypochlorite was measured. The CMCase activity of *Bacillus* strain was inhibited by SDS with I_{50} of 95 µg/ml comparable with I_{50} of *H. insolens* CMCase. The CMCase activity of *Bacillus* strain was inhibited by sodium hypochlorite with I_{50} of 23 µg/ml comparable with that from *H. insolens* CMCase. These characteristics suggest that CMCase from *Bacillus* strain may find application in laundry industry. Enzyme exhibited stability up to 80^{o} C and over a pH range of 6.0-9.0. Activation energy and enthalpy for endo-glucanse were calculated to be 19 and 25 kJ/mol respectively and indicated that endo-glucanase produced by this organism is thermos-table. Due to tolerance of purified enzyme to heat and broad pH stability, it is an attractive candidate for isolation of cellulase genes for cloning studies. The recombinant cellulases may be used in the production of fermentable sugars from biodegradable lignocellulosic materials in fuel industry.

K-3.4 Production of Thermostable Enzymes for Industry using Thermophilic Fungi

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Thermophilic fungi are well known worldwide to produce thermostable enzymes which have a visible edge on mesophilic enzyme system. The enzymes are produced in wide range of cellulases, hemicellulases, laccases, pectinases, phytases, proteases, lipases amylases, etc. All of these enzymes have applications in industries such as animal feed, textile, food, feed, leather, dairy, pulp and paper, etc. γ-Rays mutagenesis of wild type *Thermomyces lanuginosus* and mutant selection was performed. A deoxyglucose-resistant mutant of *Humicola lanuginosa* was obtained by exposing conidia to γ-rays and selected on esculin-ferric ammonium citrate agar medium containing 2-deoxyglucose (0.6%) from which colonies showing faster and bigger blackening zones were selected**.** Xylose among the monosaccharides was found to be the best enzyme inducer in mutant. Kinetic parameters for enhanced xylanase synthesis were achieved when corncobs was used as the carbon source. The combination between corncobs and corn steep liquor was the best to support higher values of all product formation kinetic parameters. Effect of temperature on the kinetic and thermodynamic attributes of xylanase production equilibrium in *H. lanuginosa* was studied using batch process. The best performance was found at 45 °C. Mutation had thermo-stabilization influence on the organism and mutant required lower enthalpy and entropy values for product formation than those exhibited by the wild organism and those of mesophilic and thermo-tolerant organisms. Biobleaching of pulp in the lab studies resulted in increase in brightness as compared to control. While kappa no. and SR^{o} was decreased significantly. *Humicola insolens* γ-Rays mutagenized spores were selected on Carboxy methyl cellulose plates using Oxgal as colony inhibitor for the selection of mutants. Colonies selected in the range of 80-120 krads, showed greater clearance of substrates using Congo red assay. Similar studies were carried out for phytase production in *Sporotrrichum thermophile* and *Thermomyces lanuginosus* that will be discussed.

K-3.5 Industrial and Environmental Application of Biosurfactant Produced by *Pseudomonas aeruginosa*

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Surfactants are capable to reduce surface tension and interfacial tension by acting upon the interfaces of two phases of hydrophobic and hydrophilic liquids, liquid-solids. Presently the huge demand for these surfactants is being fulfilled by petroleum-based, chemical surfactants. The presence and persistence of chemical surfactants in waste water has toxic effect on the environment. . *Pseudomonas aeruginosa* produces Rhamnolipids under suitable growth conditions. Biosurfactants are being looked into as possible alternatives. Biosurfactants are produced by microbes (both bacteria and fungi) and their chemical nature is well studied. These are amphiphilic compounds and being biodegradable has potential applications in various industries. They can be produced by altering the growth conditions to remain effective at very high temperature and tolerated required pH and with lower toxic effect. The Biosurfactants, having the capability of solubilizing and/or emulsifying toxic chemicals are mainly used in hydrocarbon bioremediation *in-situ* and *ex-situ.* Biosurfactants can also be applied in agriculture, detergents, products of personal care, in processing of food, cosmetics, pharmaceuticals, manufacturing of textile, laundry supplies, treatment for metal recovery, in processing of pulp in making paper and in paint industry The application of suitable biosurfactants in the process of enhanced oil recovery (EOR) as well as for heavy oils recovery is possible by many ways. Biosurfactants are also used in bakery to influence the flour rheological characteristics. In meat products these are applied for partially broken fat tissue emulsification. Biosurfactants can be applied in the pulp and paper industry, textiles, ceramics and uranium ore processing.

ORAL PRESENTATIONS

TRACK 3

3.1 Entrapment improves the stability and recycling efficiency of bacterial free α- glucosidase within microenvironment of Polyacrylamide gel

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Bacterial maltase catalyzes the hydrolysis of maltose and is known as one of the most significant hydrolyses. It has several applications in different industrial process but widely used in food fermentation technology and alcohol production. In the current study, entrapment technique was comprehensively investigated using polyacrylamide gel as a matrix support to improve the stability and catalytic efficiency of maltase for continuous bioprocesses. Maximum entrapment yield was achieved at 10% polyacrylamide concentration with 3.0 mm beads size. Optimized conditions indicated an increase in the reaction temperature from 45°C to 55°C after maltase entrapment while no change occurred in the reaction time and pH. An increase in the K_m value of entrapped maltase was observed whereas, V_{max} value decreased from 8411.0 to 6813.0 U ml^{-1} min^{-1} with reference to its free counterpart. Entrapped maltase showed remarkable thermal stability and retained 40% and 16% activity at 60°C and 70°C, respectively even after 120.0 minutes. Entrapped maltase also exhibited excellent recycling efficiency up to 08 consecutive reaction cycles. With respect to economic feasibility, entrapped maltase indicates its high potential to be used in various biotechnological applications.

3.2 Kinetic and Thermodynamic Properties of L-asparaginase Produced and Purified from *Aspergillus niger* in SSF.

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L-Asparaginase is the important and effective enzyme for the treatment of tumor cell and lymphoblastic leukemia. This enzyme was produced by *Aspergillus niger* using waste material as carrier substrate in solid state fermentation. Crude enzyme exhibited the activity of 4.45 U mL^{-1} with specific activity of 0.822 U mg^{-1} proteins. The enzyme activity and specific activity was increased to 12.04UmL^{-1} 3.981 Umg^{-1} respectively after 38-60% ammonium sulfate precipitation and it was further increased to 14.70 UmL^{-1} and 6.41 Umg^{-1} when subjected to diethylaminoethyl (DEAE) cellulose chromatography. Further purification was carried out by passing it through Sephadex G-150 column and observed increase in activity 18.8 UmL^{-1} with 8.42 Umg^{-1} specific activity. The optimum pH and temperature were recorded as 8.5 and 37 oC respectively. The *V*m and *K*m of asparaginase were derived from the Lineweaver Burke plot. Thermodynamic parameters namely *E*a, ΔH^*, ΔS^* and ΔG^* for conversion L-asparagine to L-Aspartate with asparaginase were also studied.

3.3 Isolation, Identification and Molecular Characterization of Brown Rot Fungi

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Fungi are wide group of microorganisms, holding great ecological and biological importance. Many of its species also hold a great importance in the field of biotechnology, and are employed in various processes. One of major taxonomic assembly of group basidiomycetes, are the members of "Brown rot fungi". As they are characterized as prominent fungi that is responsible for decaying of wood by degradation of lignocelluloses by its non-specific enzymes including lignocellulases and hemicellulases. Mostly fungi are identified on morphological basis but molecular identification will be helpful to get more benefits from fungi. In current study, two different brown rot fungi isolated from various locations of Islamabad, were identified on molecular basis. Collected fungi were initially identified on morphological basis by mycologist and culture on Malt Extract Agar media to get pure culture. Pure cultures were preserved on slants for future use. DNA was extracted by using isopropanol-chloroform method. Universal ITS primers were used to amplified specific sequence for the identification of fungi. PCR for amplification of DNA is followed by gel electrophoresis. Clear single band was obtained for each fungi at 727 bp and 615 bp for *Piptoporus butelines* and *Coniphora puteana*, respectively. Same sized band were also reported for these brown rot fungi in previous studies. These fungal species can be used for the treatment of wastewater especially industrial effluent and textile dyes.

3.4 Augmented Bioethanol Production from *Saccharum Spontaneum* through Enhanced Nutrient Supplementation Using Both Wild And Mutant *Saccharomyces Cerevisiae* Strains

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Due to depletion in natural energy reservoirs, technology is being rapidly shifted towards alternative ways to obtain cheaper and eco-friendly fuel and one such accredited process is bioethanol production. The current study is an effort towards improvement of bioethanol yield through optimization of fermentation parameters for mutant *Saccharomyces cerevisiae* KM-*11*. Wild *Saccharomyces cerevisiae* G-4 strain yielded 44.62 ± 0.59 (g/L) at 8 % of inoculum size, pH 4.7 and temperature 35 °C along with addition of $(NH_4)_2SO_4$ and soybean meal and EDTA to the media. Whereas, mutant *Saccharomyces cerevisiae* KM-11 yielded 94.06 ± 0.53 (g/L) bioethanol under similar conditions except for 7 % inoculum size. The production efficiency of mutant strain was more than double as compared to wild strain. Conclusively mutant *Saccharomyces cerevisiae* KM-11 has considerable potential to be used in energy sector for bioethanol production.

3.5 Production of Bioplastic using Banana Peel and its Characterization

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At present the chemical industry is searching for substitutes to decrease usage of petroleum-based non-degradable conventional plastics. In spite of their many uses and attractive properties, petroleum based conventional plastics have also many disadvantages such as production problems, landfill disposal, incineration, plastic recycling, and their adverse effect on biodiversity. Bioplastic are plastics derived from renewable biomass sources, composed of cellulose, starches, biopolymers, and a variety of other materials. In this research a starch based substrate banana peel was used and subjected to alkali and acid hydrolysis to convert it into bioplastic film. Synthesis of bioplastic was carried out in two phases. In first stage the process parameters pH and hydrolysis time was changed over a range of values. In this stage the pH of neutral range gave the best results for tensile strength analysis 0.552N/mm^2 with optimum residence time (10 minutes). In the second stage, commonly available plasticizer like glycerin, sorbitol and urea was added and compared. The sorbitol, gave maximum tensile strength (34.310 N/mm^2) with 41.434 N loads in this comparison. EDS analysis shows that K was the element present in highest concentration (6.73%) with 5.32 App concentration and 0.7918 intensity concentration in the biopolymer film.

3.6 Optimization of Critical Medium Components using Response Surface Methodology for Alkaline Protease Production by Novel strain of *Bacillus*

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A novel strain of *B. subtilis* isolated from a local Tannary of district Lahore, Pakistan was cultured in submerged fermentation for its high production of alkaline protease through Response Surface Methodology (RSM) under central composite design (CCD). The results revealed that alkaline protease production was increased by 8.9 folds using the optimized medium. The three step purification procedure yielded 24% activity with a purification factor of 8.48. The purified protease was monomeric of apparent molecular mass 15 KDa (SDS-PAGE). The catalysis of casein by alkaline protease was expressed by the Michaelis-Menten equation, suggesting that temperature and pH optima were 57˚C and 8.0 respectively and remained active within the pH range of 8-12 having half-life 37.8 min, the maximum velocity (V_{max}) was 200 IU/mL/min and the Michaelis constant was 0.090 mg/mL. The data obtained in this study may provide new insights from large-scale alkaline production by novel stain of *B. subilitis* IBL-01to industrial exploitation.

3.7 Optimization of Biodegradation Process for Disperse Textile Dyes using Brown Rot Fungi

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Textile industrial effluent is one of the major source of environmental pollution throughout the world. The current research was focused on the isolation of indigenous brown rot fungi from Islamabad. The isolated fungus was then used for the biodegradation of selected disperse dyes. The degradation process was optimized by adjusting different physical and chemical parameters with the help of Response Surface Methodology (RSM). Lignolytic enzymes including lignin peroxidase, manganese peroxidase and laccase were also studied during degradation process. Studied enzymes were also characterized for kinetic parameters as well. *Daedalea dickinsii* was successfully cultured on Malt extract agar media and identified on morphological basis. It was observed that more than 90% biodegradation achieved for different disperse dyes after day 7 at optimum conditions. There were maximum activities of lignolytic enzymes at maximum degradation of dyes, as activity reduces the degradation percentage reduce. This indicate the role of lignolytic enzymes in the biodegradation of dyes. The kinetic studies of enzymes showed the good affinity of substrates towards their enzymes. It can be conclude that locally isolated *Daedalea dickinsii* had great potential of biodegradation of disperse textile dyes by using its non-specific enzymes system.

3.8 UV-irradiation mutagenesis of *Ganoderma lucidum* for hyper production of ligninolytic enzymes

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White Rot Fungi have strong oxidative multienzyme system capable of lignin degradation and mineralization. Multienzyme system constitutes ligninolytic enzymes Lignin peroxidase (LiP), Manganese peroxidase (MnP) and Laccase which are diverse in commercial use in a variety of processes. In the current study, hyper production of ligninolytic enzymes of *Ganoderma lucidum* was based on mutagenesis using parent strain IBL-05. Mutants of *Ganoderma lucidum* were generated by exposing the inoculum to UV germicidal lamp for different time periods. Hyper producing mutants were screened and the response surface methodology was adopted to optimize conditions like pH, temperature and inoculum size, time of incubation and moisture level for enhanced production of ligninolytic enzymes followed by selection of mutants with 2 deoxy D-glucose. Central composite design (CCD) was preferred for analysis of recorded data. This design helped in analysis of effect of variables and their interactions on ligninolytic enzyme production as well as in determination of optimum conditions. According to CCD generated by response surface model P-values indicated the significance of model and its terms. Smaller the P-value greater the significance. P-value of variables was analyzed to find out that which factors are exerting more significant influence on enzyme production. The R^2 values 0.8148 for MnP, 0.6648 for LiP and 0.6871for Laccase suggested that the fitted linear, quadratic plus interactions models could explain the total variation. The predicted R^2 values for MnP, LiP and Laccase were 0.1505, -0.3209 and 0.0072. The CV% coefficient of variance values for MnP, LiP and Laccase were 8.74, 2.56 and 14.91 indicates that the model is fit for further analysis. Optimization of parameters variable yielded significant interaction and mutagenesis developed mutants with required characteristics including maximizing enzyme production. Developing mutant's technique may offer applications of UV-treatment to fungi for commercial mutant strains.

3.9 Disperse Dyes Biodegradation and Lignolytic Enzymes Production Potential of Indigenous Brown rot fungus *Coniophora Puteana* IBL-01

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Current study focuses on biodegradation of disperse textile dyes and study of lignolytic enzymes involved in biodegradation. Microorganisms like brown rot fungi (BRF) have great potential of biodegradation of dyestuffs due to their efficient nonspecific lignolytic enzyme system. In current study *Coniophora puteana* IBL-01, a brown rot fungus isolated from *Ficus carica*, was employed. The targeted disperse dyes were violet 63, orange 30, red W_4BS and red S_3B, which are widely used in textile industries. Degradation process was optimized by using Response Surface Methodology strategy. Initially degradation was observed 88% for violet 63, 80% for orange 30, 64 % for red W4BS and 59 % for red S3B on day 7. There was 4%, 7%, 16% and 17% increased in degradation observed by the addition of readily available carbon and nitrogen sources, respectively. The maximum activities observed for lignolytic enzymes were 1892.76 U/ml for LiP, 1438.45 U/ml for MnP and 834.50 U/ml for laccase. Kinetic studies of lignolytic enzymes show their efficiencies and specificities towards their substrates. The values of Km were found to be 0.751mM for Lip, 0.700 mM for MnP and 0.571 mM for laccase and that of Vmax were 1250 µM/ml/min for Lip, 1000 µM/ml/min for MnP and 1428.57 µM/ml/min for laccase. The optimum temperature was around 30 ^{0}C and optimum pH was around 6.5. It can be concluded from current study that *Coniophora puteana* IBL-01 is a potential microorganisms for the treatment of industrial effluent. It can also be used for the production of active lignolytic enzymes. Further studies should be conducted to explore its potential against industrial effluent.

3.10 Utilization of Agro-industrial Wastes for Hyper Production of Industrially Important Lipases

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Lipases are glycerol ester hydrolases that catalyze the triglycerides to free fatty acids and glycerol. The aim of this work was to produce lipases from low cost agricultural wastes i.e., corn cobs and shesham legumes. In this scenario temperature and incubation time for the lipase production was optimized by classical methods. About 154 trials were generated by applying response surface methodology along with coded and uncoded values for 8 parameters i.e., pH, moisture content, substrate concentration, inoculums size and different concentration of salts (ammonium sulphate, magnesium sulphate, sodium chloride and calcium chloride) by keeping temperature 45^{o}C for 5 days. The optimum concentration of lipase obtained by keeping the concentration of ammonium sulphate, magnesium sulphate and sodium chloride 1.75g of 0.98, 2.3g respectively while keeping the pH 5.5, inoculums size 3mL, moisture content 60.1 mL and substrate 4.8 g. The lipase activity obtained 6000 µg/mL/min by maintain the above physico-chemical parameter which also indicate its industrial potential for biodiesel production.

3.11 Synthesis of Good Quality Bioplastics using Chicken Feathers Waste

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Conventional synthetic plastics are typically the polymers of high molecular weight chemical materials which cause severe and irreversible damage to the environment. Conventional plastics do offer functionalities that are not easy to be replaced economically by any other materials but take ages to decay in nature and also produces toxins through the process of degradation. Bioplastics are biodegradable plastics produced from biological materials. Bioplastics have advantages of traditional petro-chemical based plastics but have no detrimental effects on environment and are cheaper, stronger, easier to degrade and safer to recycle. Bioplastics have a range of applications in food packaging, horticulture, manufacture of personal care items, in electronic devices and in making spare parts of auto mobiles due to their high flame retardancy and processability. Structurally, chicken feathers comprises 90% keratin having a wide network of disulfide bonds forming fibrous, insoluble structure that is tolerant towards insects, animals and microbial protease digestion makes them ideal for use in production of robust and tear-resistant bioplastics. A novel, simple, cost effective and environment responsive biological process was developed for synthesis of good quality bioplastics using waste chicken feathers as raw material. Feather powder was produced using keratin rich chicken feathers and used as key component in developing good quality bioplastics. Three types of plasticizers GL, SO, and EG were used as plasticizers and their effect on mechanical properties of resulting bioplastic were studied. EPS extracted from bacterial strain BS IBL-07. EPS was added to increase the tensile strength and biosurfactants were added to reduce the surface tension of the bioplastic. Tensile strength and water absorption determinations were carried out to characterize the resulting bioplastics. Samples with EPS as an additive and ethylene glycol as plasticizer substantially showed enhanced tensile strength and reduction in the water absorption and surface tension of the bioplastic. and among plasticizers ETG was most promising one having tensile strength of 28 MPa. Tensile strength with addition of EPS and Biosurfactant was improved to 29 MPa.

3.12 Screening and Statistical Optimization of Physio-chemial Parameters for Production of Xylanases from Agro-industrial Wastes

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Xylanases is commercially important enzyme having multiple applications in food, feed and paper industry. The present study was carried out to determine culture influence of *Fomes fomentarius* for the production of xylanases. The agro-industrial residues i.e., wheat straw, rice husk, sugar cane bagasse and siris pods was screened for high yield of xylanses. The wheat straw considered as an excellent source for hyper yield of xylanases from *F. fomentarius* while keeping the time of incubation 72h, pH 6.0 at 30 ^{o}C. The other physical and nutritional parameter was optimized with statistical design like RSM maximum xylanase activity (146±8 IU/mL) at 65% moisture content, 4 mL inoculums size, 175g Ammonium sulphate, 200mg Calcium carbonate and 1.4 g of glucose. In purification step 1.6 fold of xylanases activity was observed at 60% ammonium sulphate concentration then activity of enzyme was increased further upto 2.2 fold by purification of enzyme with Sephadex G-100 gel. The purified xylanases further characterized at optimum pH 6.0 while keeping the temperature at 40^{o}C. Xylanases showed higher specificity for oat spelt xylan with kinetic constants *Km* 1.25 mg/mL and *Vmax* 54 mM/min.

3.13 Ca-Alginate beads Immobilized Lignin Peroxidase from *Schizophyllum commune* IBL-06 has Improved Catalytic and Thermo-stability Properties

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Immobilization of enzyme is the most efficient and suitable way to impart the desirable catalytic efficiency, stability and recyclability features of biocatalysts. In this piece of study, extracellular lignin peroxidase was produced from *Schizophyllum commune* IBL-06 in pre-optimized solid state fermentation medium. The purified LiP was covalently immobilized on Ca-alginate beads using glutaraldehyde as cross linker with highest immobilization efficiency (89.41%) and 987.3 IU/mL LiP activity per bead. Scanning electron microscopy (SEM) was performed for the confirmation of enzyme immobilization on Ca-alginate beads. At optimized experimental conditions maximum decolorization of S. F. Red C_4BLN (79.6%) and S. F. Turq Blue GWWF (81.46) reactive dies was observed by using Ca-alginate beads-LiP at 30°C without hemolytic toxicity. Ca-alginate beads-LiP maintained more than 67% dye decolorization potential after three repeated cycles that gradually decreased to 15-19 % after 7^{th} runs of reusability. The immobilized LiP was found to show superior dye removal properties as compared to its free counterpart. Higher thermo stability, lower K*m* and high V_{max} features of immobilized LiP suggested its suitibility for various industrial and biotechnological applictaions. These preface findings implied that Ca-alginate-LiP can preferably be adopted for industrial effluents or synthetic dye decolorization and possibly can be further used in bioremediation of other toxic organic components.

3.14 Novel thermostability, catalytic and dye removal properties of Calcium-Alginate Bead immobilized *Trametes versicolor* IBL-04 Laccase

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Trametes versicolor is an efficient producer of ligninolytic enzymes, especially MnP and laccase under appropriate culture conditions. Laccases are extensively dispersed enzymes in nature and are blue copper-containing efficient oxidases having less redox potential. However, the industrial applications of laccases are hindered by their lower catalytic efficiencies and marginal operational stabilities under industrial process conditions. To improve their properties as industrial biocatalysts, it is mandatory to obtain laccases with enhanced operational stabilities. Extracellular laccase was produced by *Trametes versicolor* IBL-04 using corn cobs as a substrate under pre-optimized culture conditions. A 64-kDa laccase enzyme was purified and immobilized on calcium alginate beads using glutaraldehyde as a cross-linking reagent. Maximum enzyme immobilization efficiency (89%) was observed with 2-mm calcium-alginate beads that were developed using 4% (w/v) sodium alginate in 2% (w/v) calcium chloride. Immobilization of laccase enhanced the optimum temperature but caused an acidic shift in the optimum pH of the enzyme. The immobilized enzyme showed optimum activity at pH 3.0 and 60 °C as compared to pH 4.5 and 45 °C for free laccase. The kinetic constants K_m and V_{max} of laccase were significantly altered by immobilization. The affinity of enzyme toward its substrate increased (K_m decreased), leading to enhanced catalytic efficiency (V_{max} increased). Scanning electron microscopy (SEM) was performed to characterize the free and enzyme-bound immobilization matrix. Free and immobilized enzymes also were used for decolorization of the Reactive T Blue dye (030905 GWF) for three days. The free and immobilized laccases decolorized the dye by 65% and 92%, respectively, in 72 h. The immobilized enzyme retained 68% of its original activity after three cycles of repeated reuse for dye decolorization, indicating the usefulness of immobilized laccase in repeated industrial batch operations.

3.15 Production, Purification and Characterization of Glucose Oxidase from *Pleurotus ostreatus*

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Glucose oxidase/glucose aerodehydrogenase is a dimeric protein, surrounded by carbohydrate chains and catalyzes the conversion of β-D-glucose to gluconic acid with the formation of hydrogen peroxide. It can be obtained from various microbes at present and became an attractive entity because of its significance in various industries. The main objective of the study was to produce and purify glucose oxidase from *Pleurotus ostreatus* and furthermore to study its kinetic and thermodynamic parameters. The enzyme was extracted from *Pleurotus ostreatus* and purified by ion exchange and gel filtration chromatographic techniques resulting in the specific activity of 1067.42 U mg^{-1}. It was observed that glucose oxidase from *Pleurotus ostreatus* obtained a 57.43 fold purification with 68.12% recovery. The enzyme showed optimum activity at pH 6.0. Optimum temperature was observed to be 35ºC with 21.88 KJ mol^{-1} energy of activation. The Michaelis-Menton constants (K_m, V_{max}, K_{cat} and K_{cat}/K_m) were 23 mM, 16.66 U mL^{-1}, 476.17s^{-1} and 20.70$s^{-1}mM^{-1}$, respectively. The enzyme showed thermal stability at 50ºC with a half-life of 87.46 minutes. The enthalpy and free energy showed an increase with increase in temperature proved a better stability of *P. ostreatus* glucose oxidase while a highly negative ΔS* indicated very little disorderliness. It is concluded that glucose oxidase produced from *Pleurotus ostreatus* is well characterized and useful in diverse fields especially in clinical diagnostic kits.

3.16 Energy Dependent Proteolysis in Archaea

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Archaea, the third domain of life, are genotypically and phenotypically diverse collection of microorganisms which have a mutual evolutionary ancestor. There are four kingdoms of Archeae recognized so far i.e. Crenarchaeota, Euryarchaeota, Nanoarchaeota, Korarchaeota. Proteolysis plays a critical role in regulating proteins, maintaining cell growth and rapidly removing them when they are useless thus avoiding accumulation and chaos inside the cell. Protein turnover in Archaea is regulated by both energy dependent and energy independent proteolysis. Energy dependent proteolysis also known as ATP-dependent proteolysis adjusts mitochondrial biogenesis and eliminates misfolded proteins. The main players of energy dependent proteolysis are HsIUV proteasome, LON protease, ClpAP protease, FtsH protease. ATP-independent protease can either metabolize proteins or regulates protein quality to restock amino acid pool but its major flaw is that it cannot remove misfolded or unwanted proteins from the biological system as compared to energy dependent proteolysis. Energy dependent proteolysis is a more efficient way to conserve energy while inactivating/degrading unwanted protein in Archaea. Energy dependent proteolysis is time taking and accurate mechanism to get rid of or to translocate protein in Archaea.The recycling of obsolete protein and protein quality control is mainly managed by energy dependent proteolysis in Archaea.

3.17 Immobilization of *Pleurotus sapidus* WC 529 laccase on chitosan beads and its optimization and characterization

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Enzymes are the most versatile and robust biological catalysts which offer many more competitive applications in industrial processes as compared to the chemical catalysts. Production of ligninolytic enzymes by using white rot fungi through solid state fermentation of lignocellulosic material is field of interest because of its biotechnological significance. There are many applications of laccase enzyme in paper and pulp industry, food industry and textile industry. Enzyme immobilization can be defined as the attachment of free or soluble enzymes to the different types of insoluble supports which results in the reduction in mobility of enzyme. Properties of these biocatalysts are greatly influenced by selection of an immobilization strategy. In order to get fully biological active enzyme its attachment to the support must be firm without altering its functional and chemical properties. Laccase from *Pleurotus sapidus* was immobilized on chitosan beads. Different concentration solutions of chitosan in the range of 1 to 5% were tested in order to get the chitosan beads of required mechanical strength. Immobilization of laccase enzyme was found best using 2.5% chitosan solution. Different concentrations of glutaraldehyde solutions were used to treat chitosan beads in order to determine their coupling efficiency with laccase enzyme. The highest laccase entrapment efficiency of 76% was found using 2% glutaraldehyde solution after six hours of incubation. Kinetic parameters, K_M and Vmax values, were determined respectively as 114µM and 370 U/mL for free enzyme at pH 3 and temperature 40°C. While K_M and V_{max} values were 100µM and 580 U/mL for immobilized laccase at pH 8 temperature 60°C. The operational and thermal stabilities of the immobilized laccase were improved compared to free counterpart. The immobilized laccase enzyme was also used for the decolorization of reactive dyes from aqueous solution. The laccase immobilized on chitosan beads was very effective for removal of textile dyes from aqueous solution which creates an important environmental problem in the discharged textile dying solutions.

3.18 Hyperactivation and thermostability enhancement of *Ganoderma lucidum* IBL-05 LiP Immobilized using Sodium Alginate

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The immobilization of enzyme is one of the key issues both in the field of enzymatic research and industrialization. Among the several methods of immobilization, entrapment in Ca-alginate beads is one of the easiest and economic way to improve biocatalytic behavior of enzyme. Partially purified lignin peroxidase (625.8IU/mL) produced from *Ganoderma lucidum* IBL-05 is entrapped in Ca-alginate beads. Immobilization of LiP is carried out by varying the concentration of sodium alginate solution (1-5% (w/v)) and characterized by using veratryl alcohol as a substrate. The highest immobilization yield (70.7%) was noted at 4%(w/v) sodium alginate. Temperature changes from 25 ◦C to 50 ◦C and pH changes from 3 to 6 before and after immobilizatioin of lignin peroxidase. Reaction time for immobilized enzyme assay was also increased. Enzyme activity decreased when the concentration of alginate was increased above 4%. Immobilized enzyme retained its activity for longer time and can be reused upto three times. The enzyme activity, thermostability, reuseability and storage stability of entrapped Lignin peroxidase is found to be increased after immobilization.

3.19 Characterization of Alkali Insoluble Carbohydrate Polymers from Various Parts of Desert cotton

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It is necessary to evaluate the influence of alkali on polysaccharides composition of medicinally important plant materials. To investigate the characteristics properties of polysaccharides in Desert cotton after different levels of extraction. Various parts of desert cotton was sequentially extracted to obtain alkali insoluble polysaccharides and analyzed by FTIR spectroscopy, XRD and spectrophotometric methods for iron binding capacity. Results – FTIR, XRD and iron binding capacity of alkali insoluble polysaccharides reveals that FTIR spectra in the 1200-800 cm^{-1} give information about main polysaccharides present in polysaccharides mixtures. Leaves, stem and root extract of desert cotton spectra display a series of narrow absorption bands, typical of crystalline polysaccharide. Absorption bands at 865.82 cm^{-1}, 871.22 cm^{-1}, 868.61 cm^{-1} for galctopyranose and arabinofuranose are due to ring structure and C-OH group vibration to the spectral shape which is due to overlapped band at 868-880 cm^{-1} comparable to our result. The band at 1417.91 cm^{-1}, 1339.28 cm^{-1} 1418.10 cm^{-1}, 1339.22 $cm^{-1,}$ 1417.45 cm^{-1}, 1339.41 cm^{-1} represent C-H, OH stretching in polysaccharides such finger prints are characteristic of homogalactronic acid (except rahmnogacturanan-I or arabinogalactan). Significant effect of alkali was observed on polysaccharides of Desert cotton at various levels analysis in various parts of plant. It suggests that alkali exposure during extraction and analysis increases distinguishing polysaccharides of plant materials.

3.20 Comparison of free and immobilized laccases produced by *Pleurotus ostreatus* IBL-02

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Laccase is a unique, environmentally friendly and efficient biocatalyst that can degrade pollutants and also have diverse industrial applications. However, the industrial and environmental applications of laccase are considerably limited by its susceptibility to environmental changes and its poor reusability. To overcome this problem, laccase from *Pleurotus ostreatus* IBL-02 was immobilized on Ca-alginate beads using glutaraldehyde as cross-linking agent. Different concentrations of Na-alginate, $CaCl_2$ cross-linking agent were optimized. Best results were obtained at 2% Na-alginate, 0.5M $CaCl_2$ solution and 0.2 % glutaraldehyde. The activity of the free and immobilized enzymes as a function of pH, temperature, storage stability, kinetic parameters and periodic use were also compared. The immobilized enzyme showed good storage stability. The *K*m and *V*max values were altered after immobilization. The *K*m values for Ca-alginate was low compared to the free enzyme. This may be due to the fact that immobilization on Ca-alginate in presence of $CaCl_2$ exposed certain active sites of the laccase.

POSTER PRESENTATIONS

TRACK 3

3.1-P Process Optimization for Bioethanol Production from Cellulosic Waste Paper Fermentation by Exploiting Cellulolytic Bacteria Isolated from *Microtermes obesi* Gut

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The purpose of this study was to produce an ecofriendly biofuel (bioethanol) from cheap urban cellulosic waste office paper by utilizing cellulolytic bacterial isolates from gut of local termite specie *Microtermes obesi* to overcome the energy problems. Carboxymethylcellulose (CMC) was used to isolate cellulolytic bacteria from termite gut. MO-1 was selected as highly potent isolate on the basis of halozones formed during primary screening and glucose production during secondary screening. To provide suitable conditions for maximum growth and cellulolytic activity, MO-1 was standardized at different physiochemical conditions. Maximum growth along with maximum glucose yield was obtained at optimum temperature 37°C, pH 7.0, substrate concentration 5 % (w/v), 6 % bacterial inoculums and agitation at 150 rpm. Growth of cellulolytic bacteria along with glucose yield was enhanced when 0.1 % PEG and Ca^{2+} ions were added to the media. Through Simultaneous Saccharification and Fermentation (SSF) method *Saccharomyces cerevisae* was utilized for conversion of glucose produced by saccharification of waste office paper into bioethanol. The yield of ethanol obtained by saccharification of office paper by MO-1 was 4.81 mg/ml. According to 16S rRNA sequence homology, the bacterial isolate MO-1 was identified as *Mesorhizobium ciceri.* The data gathered in the current study revealed that cellulolytic bacteria *Mesorhizobium ciceri* MO-1 isolated from the gut of *Microtermes obesi* was competent enough to breakdown the cellulosic content of waste office paper into glucose monomers which was later be fermented into bioethanol. This study was also beneficial with respect to bioremediation of urban waste (office paper).

3.2-P Dye Decolorization and Detoxification Potential of Ca-alginate beads Immobilized Manganese Peroxidase

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A promising and eco-friendly novel entrapment approach was adopted to immobilize *G. lucidum* IBL-05 MnP on Ca-alginate beads. The immobilized MnP was subsequently used for enhanced decolorization and detoxification of Sandal reactive dyes to explore its environmental and industrial applicability. Ca-alginate bound MnP was catalytically more vigorous, thermo-stable, reusable and worked over wider ranges of pH and temperature as compared to its free counterpart. Managese peroxidase (MnP) from *G. lucidum* IBL-05 was purified and immobilized. Thepotential of free and Ca-alginate immobilized MnP for decolorization and detoxification of new class of reactive textile dyes (Sandal Reactive dyes) was investigated. MnP isolated from solid-state culture of *Ganoderma lucidum* IBL-05, presented highest immobilization yield (83.9%) on alginate beads prepared using optimum 4% (w/v) sodium alginate, 2% (w/v) $CaCl_2$ and 0.5 mg/ml glutaraldehyde. The immobilized MnP showed optimum activity at pH 4.0 and 60°C temperature as compared to pH 5.0 and 35°C temperature for free enzyme. The kinetic constants K_m and V_{max} of MnP were significantly improved by immobilization. The enhanced catalytic potential of of immobilized MnP led to 87.5%, 82.1%, 89.4%, 95.7% and 83% decolorization of Sandal-fix Red C_4BLN, Sandal-fix Turq Blue GWF, Sandal-fix Foron Blue E_2BLN, Sandal-fix Black CKF and Sandal-fix Golden Yellow CRL dyes, respectively. The immobilized MnP was reusable for 7 repeated cycles in dye color removal . Furthermore, immobilized MnP also caused a significant reduction in BOD (94.61-95.47%), COD (91.18-94.85%) and TOC (89.58-95%) of aqueous dye solutions. Results of Cytotoxicity tests like hemolytic and brine shrimp lethality tests suggested that Ca-alginate immobilized MnP may effectively be used for complete detoxification of dyes and industrial effluents.

3.3-P Decolorization of reactive dyes using Laccase from *Trametes versicolor* IBL-04 immobilized on PVA-alginate beads

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Polyvinyl alcohol (synthetic polymer) has good mechanical strength rather than calcium-alginate which can easily be biodegraded. PVA-alginate immobilized enzyme extremely good characteristics, therefore this technique suitable for entrapment of Laccase. Monomeric 66kDa Laccase produced by *Trametes versicolor IBL-04* in SSF of corncobs (911U/mL) was purified through chromatographic techniques. Immobilization of enzyme PVA–alginate beads was used as a carrier for laccase immobilization. The maximum enzyme immobilization efficiency of 76.41% was observed by using 12 % PVA concentration with 2 % alginate. PVA-alginate has a good ability to recover the strength and toughness of the beads with 2.5 mm bead size. This micrograph specified that inside the PVA-alginate microspheres entrapment had been formed with the help of coupling agent (glutaraldehyde). The structure and morphology of the modified samples showed by SEM that beads with immobilized laccase on the surface were spherical in shape having large surface area. Unbound free enzyme more reactive at pH 5 have enzyme activity 810 U/mL with 100% relative activity at 45^{o}C and Laccase immobilized through PVA-alginate showed maximum enzyme activity 973U/mL at pH 6.0 mL with 100% relative activity at 70^{o}C . The PVA-alginate immobilized enzyme exhibited greater pH and thermal stability at high temperature. The PVA-alginate beads immobilized Laccase had lower *K*m (12μM) and *V*max 962µM/min values as compared to its soluble counterpart thus indicates high affinity of enzyme for its substrate. Storage stability of Laccase immobilized by PVA-alginate, retained the maximum 79% residual activity as compared to free enzyme have 32.1% at the end of month after 28 days at 4^{o}C . The immobilized laccse showed high decolorization efficiency (98.04%) for S. F. Black CKF in 24 h. The immobilized enzyme retained 50% of its original activity after seven consecutive cycles of repeated reuse for dye decolorization, indicating the usefulness of immobilized laccase in repeated industrial batch operations. The kinetic and thermo-stability characteristics of PVA-alginate beads immobilized laccase reflect that the enzyme has potential for use in industrial and environmental biotechnology.

3.4-P Disperse Textile Dyes Biodecolorization By Selected Brown Rot Fungi And Analysis Of Enzymes Involved In Underlying Process

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Current study was designed to investigate the ability of *Coniophora puteana* IBL-01, *Daedalea dickinsii* IBL-02, *Piptoporus betulinus* IBL-03 and *Fimitopsis pinicola* IBL-04 to decolorized disperse textile dyes. Five disperse dyes, purchased from supplier were studied including; disperse violet S3RL, orange S2RFL, red W4BS, yellow SRLP and red S3B. The decolorization process was observed for 10 consecutive days with the analysis of process on each day. The results showed that *Daedalea dickinsii* IBL-02 (70-80 %) has the more potential of biodegradation of disperse dyes while *Piptoporus betulinus* IBL-03 (47-59 %) has the least. The study of lignolytic enzymes i.e. lignin peroxidase, manganese peroxidase and laccase showed that *C. puteana* IBL-01 and *D. dickinsii* IBL-02 produced most active enzymes. Higher enzymatic activities related with more degradation indicated that these are involved in decolorization process. Enzymes showed maximum activities at 30 ^{0}C and pH 6.5 with good affinity towards their substrates as indicated by kinetic values. This study will bring the attention of other scientist to use brown rot fungi for the treatment of wastewater.

3.5-P Exploration of Optimum Operating Conditions for Enhanced Laccase Production by *Pleurotus nebrodensis* WC 850 through Response Surface Methodology

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Laccase is a blue copper containing enzyme which is capable of catalyzing the oxidation of phenolic compounds to phenoxyl radicals. The purpose of recent study was to find optimum conditions for maximum laccase enzyme production by *Pleurotus nebrodensis* WC 850 by applying an important statistical technique response surface methodology (RSM) through solid state fermentation of lignocellulosic biomass. Firstly, screening experiments were conducted in order to select the best substrate for maximum laccase enzyme production among six different lignocellulosic substrates. Wheat straw was selected as best substrate with enzyme activity 145.10 U/mL after 6^{th} day of fermentation. Different physical parameters including pH, temperature, inoculum size, incubation time and moisture were then optimized by response surface methodology under central composite design (CCD). RSM is an effective strategy which explores the optimum operating conditions for multivariable system as well as it also examines the simultaneous, systematic and effective variation of crucial components and determines the possible interaction among higher order effects which results in enhanced enzyme yield. The increase in laccase enzyme activity (171.63 U/mL) was observed at pH 5, temperature 30^0C, inoculum size 4mL, incubation time 144mL and moisture 50% after optimization of different factors at different levels. This study revealed that optimization of different factors have significant effect on laccase enzyme production. Moreover, optimization of different parameters through RSM is time saving and good technique in biotechnology.

3.6-P Improved thermostability, catalytic and Reusability Properties of Chitosan Beads Immobilized *Schizophyllum commune* IBL-06 Lignin Peroxidase

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The lignin mineralizing enzymes (LMEs) system of white rot fungi (WRF) has tremendous catalytic prospect of oxidative bioremediation of a number of toxic compound pollutants and many other industrial uses. Immobilization enables the reuse of enzymes and making them industrially relevant and economical biocatalysts. *Schizophyllum commune* IBL-06 was cultivated for the secretion of lignin peroxidase (LiP) in pre-optimized solid state fermentation medium of corn Stover. A high yield of lignin peroxidase production (1347.3 UmL^{-}) was recorded in crude culture supernatant. The enzyme was purified (3.1 fold) by a pre-standardized four step protocol comprising ammonium sulphate fractionation, dialysis, DEAE cellulose ion exchange and Sephadex G-100 column chromatography. The 38 kDa single polypeptide *S. commune* IBL-06 LiP migrating as a single clear band on both native and sodium dodecyl sulphate polyacrylamide gels. The purified enzyme was then immobilized on chitosan beads using glutaraldehyde as activating/cross linking agent. Scanning electron microscopy (SEM) was performed for the confirmation of enzyme immobilization on chitosan beads. The highest textile dye decolorization (95.45%) potential was observed with chitosan-immobilized enzyme at 30°C without hemolytic toxicity. The chitosan beads-LiP retained approximately more than 70% activity after three repeated runs that gradually decreased to 20 % after 7^{th} cycle of reusability. The immobilized LiP was found to show superior dye removal properties as compared to free LiP. Higher thermo stability, lower K*m* and high V_{max} features of chitosan beads immobilized LiP suggested its suitibility for various industrial and biotechnological applictaions.

3.7-P Screening, characterization and optimization of potential Bioplastics (PHA) producing bacteria isolated from soil and industrial waste

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Bioplastics or Polyhydroxyalkanoates (PHAs) are biodegradable plastics and provide a sustainable strategy against persistent synthetic plastics. In this study, isolation and purification of PHA producing bacteria from agricultural soil and industrial wastes was done by using PHA detection agar (PDA). Total 24 isolates were obtained. Thereafter, Screening of PHA producing bacteria was done through Nile blue staining and Sudan black staining. Extraction of PHA was done by using sodium hypochlorite digestion method. Finally, optimization for PHA production was done by optimising nitrogen concentration, pH and temperature. Growth and PHA production ability was investigated under different growth conditions using different strains, selected on the basis of their PHA yield showed that out of 4 best producers strains, 2 strains were found to be the most active PHA producers as they produced high PHA percentages *i.e.* 25.95 and 19.04%.

3.8-P Comparative assessment the metal bioaccumulation rate in gills, liver, kidney and muscle tissues of freshwater fish, *Cirrhinus mrigala* collected from River Ravi by oxidative stress biomarker catalase

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At the present time, metal pollutants in the aquatic ecosystem has become one of the major problem for aquatic life due to their involvement in oxidative radicals production. Fish possess oxidative reluctant guard system called antioxidant in all vital body parts that defends them against metal generated toxic oxidative radicals. Catalase (CAT) is an important part of antioxidant defense system that involves in catalyzing thousands of oxidative/reductive reactions in living systems and is considered as a good biomarker for the presence of pollutants in aquatic ecosystem. The purpose of present study was to compare the level of catalase enzyme and selected heavy metals i.e. Pb, Cd, Zn, Cr and Co in gills, liver, kidney and muscle tissues of *Cirrhinus mrigala* collected from main fishing stations (Balloki Headworks and Sidhnai Headworks) of River Ravi, Punjab, Pakistan. The inferences of metal accumulation and catalase enzyme activity found in studied body parts of riverine fish were also compared with fish collected from pound water. The results showed lower catalase enzyme activity in all studied body parts of riverine fish as compared to pond fish. Statistical analysis showed significant difference ($p \leq 0.05$) between wild captured and pond raised fish studied body parts catalase enzyme activity. The metal bioaccumulation rate measured in order as liver > kidney > gills > muscle in fish collected from Balloki Headworks while, liver > kidney > muscle > gills in fish collected from Sidhnai Headworks. The metal accumulation rate in pond fish was observed negligible and only Zn was noted in trace amount. On the basis of present study, it is concluded that catalase enzyme activity in different parts of fish can be used to predict the presence of pollutants in aquatic ecosystems which will be helpful in making preventive measure against toxic pollutants.

TRACK 4

FOOD, NUTRITIONAL

AND

HEALTH BIOCHEMISTRY

TRACK COMMITTEE

1. **PROF. DR. TAHIRA IQBAL (FOCAL PERSON)**
2. **DR. MUHAMMAD SHAHID**
3. **DR. YASAR SALEEM**

KEYNOTE LECTURES

TRACK 4

K-4.1 Novel Aptamer Coupled Fluorescent Nanoparticle Assay for Multiplexed Food Pathogen Detection

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Foodborne pathogens cause serious human infections annually as they are ingested unnoticeably due to low quantities. Therefore, there is a direct need for efficient, fast, cost effective and reliable pathogen detection methods which can identify trace amounts of the pathogens simultaneously. Advances in selection of pathogen specific synthetic aptamers, have provided us with the opportunity to develop highly efficient and specific biosensors. Aptamers have several advantages over antibodies, such as cost effectiveness, small size, thermal and chemical stability, *in vitro* selection ability and ease of labelling. In recent years, coupling of aptamers with fluorescent nanoparticles have lead to rapid detection food pathogens as well as many other molecules from ions to disease biomarkers. However, only one type of nanoparticle has been used in these multiplexed assays. Here we report a model multiplex detection protocol based on the aptamers coupled with two different fluorescent nanoparticles. *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* specific ssDNA aptamers were coupled with carboxylated Up-converting nanoparticles and Quantum Dots facilitated by carbodiimide chemistry. The resulting nanoparticles were conjugated with the magnetic beads that were previously coupled with the corresponding half-complementary DNAs. These conjugates were used as the molecular recognition elements for the simultaneous detection. Two different laser excitations were used to measure the change/reduction in the fluorescence signal of the nanoparticles, following the incubation of the conjugates with the bacteria. The system was able to detect significantly low number of different pathogens, suggesting a convenient multiplexed analyte detection strategy also for analytes rather than the bacteria.

K-4.2 Mineral content of Pakistani foods: an update of food composition database of Pakistan through indirect method

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Analysis of nutrients to develop or update food composition database is costly and time consuming. Alternative approach is to add the data on nutrients from existing literature using guidelines set by International Network of Food Data Systems (INFOODS). Current food composition database of Pakistan is available in table form and is many years old, therefore, need to be updated. Here, mineral content on various foods from Pakistan was collected from existing literature and scrutinized against a set for inclusion in updated food composition database of Pakistan. Minerals data for foods of Pakistan was updated with eight minerals (Ca, Fe, Zn, P, Mg, K, Na and Cu) for 37 food items. Additional information (Cr, Co, Cd, Pb, Ni and Mn) was also updated for many foods. Further, selected mineral data (Fe, Zn, P and Ca) was compared with food composition table of Pakistan (revised in 2001) to check the variations between the two with the passage of time. It was observed that mineral content deviate positively and negatively for most of the foods. Current study suggest that updating food composition databases thorough indirect method is feasible option when good quality scientific reports on number of foods are available for country.

K-4.3 Hepatitis C Virus Infection: An Emerging Risk Factor for Thyroid Disorders in Pakistan

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Pakistan is one of the top three countries of world where prevalence of Hepatitis C virus (HCV) infection is alarmingly high (4.8%). HCV primarily causes hepatic complications but chronic HCV infection is also related to a large number of extrahepatic manifestations including autoimmune thyroid disorders. As thyroid disorder (TD) and thyroid autoimmunity (TA) are more prevalent in female gender we compared incidence of TDand TA between female HCV positive patient before interferon treatment (n=164; Patient group) and healthy HCV negative women (n=223; Control group) at CENUM, Mayo Hospital Lahore. Serum free thyroxin (FT_4) and thyroid stimulating hormone (TSH) were detected by radioimmunoassay (RIA) techniques. Serum thyroid peroxidase antibodies (TPO-Ab) titer was determined by ELISA method using commercial kits.The age range of patients and control group was 10 to 60 years. Serum TSH was abnormal in 24 (14.6%) patient and 26 (11.6%) control women but the difference was not significant (p=0.188). Similarly incidence of TD was 8.5% and 4.0% respectively with no significant difference (p=0.691). Serum TPO-Ab was determined in 140 patient and 162 control women with normal thyroid function status. Among HCV patients 39 (27.8%) and among control 14 (8.6%) women were TPO-Ab positive. The difference was statistically significant (P<0.001). The TPO-Ab positivity in more than one fourth of local untreated HCV female patients is alarming as these patients are at greater risk of developing thyroid disorders either during and/or after interferon treatment. Thus HCV infection is posing a threat to future thyroid health of Pakistani female.

K-4.4 *In vitro* effect of salt stress on regeneration and carbohydrate metabolism in basmati rice

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Rice is an important food crop that serves as a primary source of energy for mankind around the world. Many biotic and abiotic stress factors hamper its maximum yield potential. Among these, salinity is a major environmental factor which effects its growth and productivity. Tissue culture is an important technique for the development of salt tolerant genotypes. Proposed research work was designed to determine *in vitro* effects of salinity stress on growth and carbohydrate metabolism in basmati rice. Super basmati showed highest callus induction frequency (95%) on MS medium supplemented with 3mg/L 2,4-D followed by basmati 5015(92%) and basmati 515 (90%). After callogenesis, calli were shifted on regeneration medium containing NaCl (0mM, 50mM, 100mM and 150mM concentrations). Super basmati performed better than other varieties with highest regeneration efficiency of 92% followed by basmati 5015 (89%) and basmati 515 (85%). Salinity stress effected regeneration efficiency and caused reduction in leaf relative water contents in all varieties. Super basmati tolerated salt stress as compared to other varieties by maintaining higher proline contents with increasing stress level. Accumulation of total soluble sugars and sucrose was observed in basmati 515 at higher level of stress whereas their concentration in super basmati remained the same as control. In response to salinity stress the activity of sucrose phosphate synthase and alkaline invertase was reduced in basmati 515 whereas increased in Super basmati. This increased enzyme activity was responsible for higher starch accumulation. Although it is unclear whether starch accumulation may play an important role in salt tolerance cultivar, it is possible that adjusting carbon partitioning and allocation could have an important implication on overall plant growth under salinity.

K-4.5 Evaluation of Nutritional Quality In Meat Of Three Farmed Carp Fish Species (*Labeo Rohita, Cirrhinus Mrigala, Catla Catla*) Raised Under The Same Conditions

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Analysis of fatty acid (FA) composition in freshwater fishes promotes understanding of the potential relationship between fish health or human nutrition and specific FA's. It is well known that fish lipids are effective against cardiovascular diseases due to their high contents of long chain polyunsaturated omega-3 (*n*-3) fatty acids. Therefore, the chemical identity of FA's in endemic fishes must be established. This study was carried out to determine the proximate composition, fatty acid profile and biometric data for farm fish species *Labeo rohita, Cirrhinus mrigala,Catla catla* raised under identical conditions. The analyzed fish species were found as source of high polyunsaturated fatty acids with low fat high protein. Fat content of the fish fillets was in range 2.57–3.11 g/100 g. Whereas fat content for feed was 14.90 g/100 g. The protein level for the three fish ranged from 20.00–23.57 g/100 g and in feed it was estimated in the range of 67.70 g/100 g. The palmitic acid and stearic acid were the main saturated fatty acids (SFA), oleic acid was the predominant MUFA while the docosahexanoic acid and eicopentanoic acid were the major PUFA. The percentage of DHA exceeded that of EPA in all fish species analysed. The n_3/n_6 ratio ranged from 1.69–1.91. PUFA/SFA ratio was much higher in L. rohita (1.40) due to abundance of n_3 PUFA, particularly DHA.

ORAL PRESENTATIONS

TRACK 4

4.1 The Associations between Serum Resistin and 25-hydroxyvitamin D Levels in Saudi Diabetic Patients

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The aim of this study was to investigate associations between serum levels of an adipokine namely; resistin, and 25 – hydroxyvitamin D in Saudi diabetic patients. One hundred and eleven diabetic subjects were recruited for the cross-sectional study at King Fahad Medical City, Riyadh, Kingdom of Saudi Arabia. Blood samples were analyzed for biochemical parameters. Serum resistin level and D were measured for the participants including those who are diabetics and at high risk to develop atherosclerotic cardiovascular diseases. Out of the 111 diabetics 56 were type-1 and 55 type-2. Serum resistin level in control group was 14.41 ± 11.5µg/ml compared to 20.21 ± 16.94 µg/ml in diabetic group ($P \leq 0.001$). For type-2 diabetes mellitus it was19.53± 17.35 µg/ml whereas in type–1 diabetes mellitus it was 25.22 ± 14.82 µg/ml P = 0.393. Vitamin 25 (OH) D level in diabetic subjects was 50.67 ± 35.6 nmol/L whereastype -2 was 51.67±36.90nmol/L compared to 48.4±34.5nmol/L for type -1.The level of resistin in those taking vitamin D supplementation was 21.34 ± 18.27µg/ml; and the resistin level in those not taking vitamin D supplementationwas 17.4±11.19 µg/ml; (P =0.237. No association was found between resistin level and Vitamin 25 (OH) D in Saudi diabetic patients. Blood urea and creatinine showed significant correlation with resistin level in one hand and Vitamin 25 (OH) D on the other hand. Several in vitro and in vivo studies have confirmed that adipoikneresistin have numerous important functions in the body. The circulating resistin levels and Vitamin 25 (OH) D were associated with general inflammation in renal diseases. This study showed no significant correlation was found between resistin level in diabetic patients and Vitamin 25 (OH) D in type 1 and type -2 diabetes mellitus. The correlation of resistin and vitamin D with urea may reflect their involvement in kidney dysfunction. The level of serum resistin showed slight increase in diabetic patients compared to control group and it was higher in type -1 diabetes mellitus. More efforts are needed to explore the physiological mechanism of resistin action in metabolic disorders.

4.2 Irradiation As A Quarantine Treatment To Prevent Post Harvest Spoilage Of *Daucus Carota*

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The carrot (*Daucus Carota* L.) has a remarkable role in the human diet since old times. No doubt it holds fascinating vitamins, minerals and essential nutrients that are required for the growth and development of a healthy human being. The postharvest diseases are considered worldwide as the major issue for postharvest facilities. Although there are various methods to decrease postharvest losses, consumers are looking for agricultural product free of chemicals. Gamma radiation is a commercial method to prevent such losses. In the present study effect of gamma irradiation on carrots were observed, for the evaluation of nutritional value and extension of shelf-life. The samples were subjected to various irradiation doses 1.0, 1.5 and 2.0 kGy kept at refrigerated temperature up to 21 days. Sensory properties, microbiological and physical analysis was carried out on weekly intervals for the treated and untreated samples. Present study revealed that bacterial, mold and yeast count was reduced in all irradiated carrots as compared to that of control. The microbial flora eliminated to maximum extent at dose 1.5 kGy. And the samples receiving the dose of 2.0 kGy were decayed completely within one week.

4.3 Purification, biochemical characterization and homology modeling of Antifungal protein from seeds of *Caesalpinia bonducella*.

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An antifungal protein, chitinase from seeds of *Caesalpinia bonducella* was purified to electrophoretic homogeneity by combination of ammonium sulphate precipitation, size exclusion and ion exchange chromatography. The purified chitinase gave a single band of molecular mass of 26 kDa on SDS-PAGE under reducing condition, indicating that the enzyme was a monomer. The enzyme showed maximum activity at pH 5.5 and at a temperature of 30^{o}C. The Km and Mmax were 2.1 mM and 613 U/min/mg of protein respectively. The enzyme was strongly inhibited by Hg^{+}, Hg^{2+} and *p*-chloromercuribenzoic acid and moderately activated by 2-mercaptoethanol. The N-terminal amino acids sequence and MS/MS data analysis of the enzyme showed high degree of sequence similarity to chitinases reported from Papaya and *Leucaena leucocephala*. Homology model of the enzyme was constructed using crystal structure of Papaya Chitinase as a template.

4.4 Gamma Irradiation Of Sheep Meat: Taking Food Safety To Next Level

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Sheep (*Ovis aries)* is well-thought-out to be a significant source of nutrition around the world. Basically this study is about exploiting the possibility of irradiating sheep meat with Gamma Radiation and optimizing a radiation dose that can be set as a standard against which sheep meat could be sterilized in future in Pakistan. Approval for meat irradiation for monitoring microbial load came in February 2000. General public can be protected from foodborne ailments by the use of Gamma irradiation technique, which remains an underutilized but indeed a very fruitful technology. Pathogenic microorganisms have caused numerous outbreaks of foodborne illnesses in many countries in the past in spite of the mighty efforts their government employed to avoid contamination. Appropriate hygienic practices can lower the level of contamination but major problem at the moment is the form in which meat is sold i-e raw. In such cases, it becomes increasingly impossible to eliminate these important pathogens from farms and primary processing is also of no help to the case in current circumstances. A number of decontamination methods are practiced nowadays but the most adaptable option among them is to process the pathogens with ionizing radiation. It is an efficient, safe, environmental friendly and energy efficient process. Irradiation emerges out to be more treasured as an end product sterilization procedure. Depending on conditions of irradiation and the food, radiation treatment at doses of 2–7 kGy can adequately eliminate possibly pathogenic bacteria that includes both long-time known pathogens such as *Salmonella* and *Staphylococcus aureus* as well as new emerging pathogens such as *Campylobacter jejuni, Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products without causing any changes in nutritional, sensory and technical qualities. Decontamination process is used today mostly for poultry and red meat, egg products and fishery products.

4.5 Association of Single Nucleotide Polymorphism of Interleukin 6 Receptor Gene With Rheumatoid Arthritis in Patients From Pakistan

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Increased production of interleukin 6 (IL-6) is associated with rheumatoid arthritis that acts through its receptor, IL-6R (Interleukin 6 receptor). Various single nucleotide polymorphisms in IL6R gene confer susceptibility to rheumatoid arthritis have been identified in various population yet these associations have not been not fully established. The present study was pursued with the aim to evaluate a possible association between three single nucleotide polymorphisms (*rs2228145, rs4537545, rs4845617*) of *IL-6R* gene and rheumatoid arthritis in Pakistani patients. For this purpose, we recruited 60 patients diagnosed with rheumatoid arthritis and 60 healthy age and gender matched controls. Blood samples were collected and DNA was extracted. Sanger DNA sequencing was performed to evaluate the SNPs in *IL6R* and the data were statistically evaluated using chi-square test. Results of chi-square test of independence showed that allele C of *rs2228145* was significantly associated with rheumatoid arthritis in patients (Odds ratio or OR= 6.78, chi-square or χ^2=43.26, p=0.0001). Moreover, the G allele of *rs4845617* was also significantly associated with rheumatoid arthritis in patients (OR=2.28, χ^2=8.98, p=0.0027). However, we could not find any association between *IL6R* (*rs4537545)* with rheumatoid arthritis in Pakistani population. Our results signify that these polymorphisms may be associated with rheumatoid arthritis susceptibility in Pakistani patients.

4.6 Assessment Of Major Foliar Blight Associated With Maize Crop From Bhimber Azad Kashmir

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Maize is the second staple food in Azad Kashmir. In the recent analysis, we isolated, measured and control the *Zea maize* major foliar blight fungal diseases from three tehseels of district Bhimber Azad Kashmir. The foliar blight diseases viz., Southern Corn Leaf Blight (SCLB), Northern Corn Leaf Blight (NCLB), *Phaeospheria* Leaf Spot (PLS) and *Curvularia* Leaf Spot (CLS) were found prevalent in different localities of Bhimber Azad Kashmir. The blight incidence, distribution and severity calculated and their remidies were absorved to improve the corn quality. It was observed that percentage disease incidence and disease severity of foliar corn blight (SCLB, NCLB, PLS and CLS) increased at mature stage as compared to young and seedling stage, respectively. So diseases play a significant role in reducing the maize production. Pathologists try to increase the yield through minimizing the losses particularly due to foliar blight diseases in maize. The researchers made a significant contribution by developing disease resistant varieties and crop rotation. There were seven species of mycoflora associated with maize seeds in the analyzed samples. On infection rate basis *Aspergillus niger* (52.5%) and *Alternaria alternata* (46.0%), respectively. To reduce or eliminate the detrimental impacts of these species, four different management strategies were evaluated in experimental plot and results were analyzed by LSD. The garlic extract treatment was the best with highest seed germination rate (57.48%), followed by distilled water treatment (48.88%), fungicide treatment (37.18%), and hot water treatment (35.96%), respectively. It was observed that all the results were significantly different from each other but the interaction between treatments and localities showed various degrees of variations.

4.7 Antioxidant Attributes, Phenolics Acids Composition And Biological Activity Of Extracts From Japanese Persimmon (*Diospyros Kaki*) As Effected By Organic Solvents And Drying Technique

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The present study was carried out to investigate the effect of solvents extraction and drying techniques on the antioxidant activity and biological activity of persimmon (*Diospyros kaki*). The samples of fresh, sun dried and shade dried persimmon were taken to examine the antioxidant activity of their peels and pulps. The crude extract yields from the persimmon samples ranged from 11.1-84.8% of dry weight of pulp and 17.91-62.9% for peel. Total phenolic contents (TPCs), total flavonoids contents (TFCs), DPPH radical scavenging and reducing power assay were used for determination of antioxidant activity. Bacterial activity was done by disc diffusion method against four selected bacterial strains. Individual phenolic acids were analyzed by HPLC. TPCs and TFCs values for peel and pulp extract varied from 31.8-43.08 GAE mg/g DW, 22.3-35 GAE mg/g DW and 16.31-27.29 CE mg/g DW, 13.36-17.9 CE mg/g DW, respectively. The persimmon peel and pulp exhibited good DPPH radical scavenging activity and reducing potential with contribution of IC_{50} 0.63-2.09, 1.08-2.55 mg/mL and EC_{50} 6893.20-12476.71 and 15723.10-21161.20 mg/mL of extract, respectively. Total amount of individual phenolic acids in persimmon extracts varied 20.9-553.15 mg/Kg of extract. The Persimmon extracts exhibited the good antimicrobial activity against selected bacterial strains. With regard to different aqueous organic solvents used 80 % methanol was found to be most effective solvent for the extraction of bioactive compounds. So, the present findings suggested the use of persimmon peel and pulp as a valuable resource of antioxidants thus can be used in functional food and pharmaceutical industry.

4.8 Microbiological analysis of ready-to-eat salads available at different outlets in Lahore, Pakistan

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Food-borne diseases are the global public health problem. These play a significant role in human morbidity, mortality and economic loss. Ready-to-eat (RTE) salads are considered as a high-risk food because they do not require any heating, washing or cleaning prior to consumption. Therefore, we aimed to determine the microbiological quality of RTE salads in our locality. A total of 50 different salads were collected aseptically from different vendors and restaurants of Lahore, Pakistan. Each sample (10 g) was homogenized in stomacher. The homogenized material was serially diluted up to 10^{-6} using 0.1% peptone water as diluent. The dilutions were inoculated on blood, nutrient and MacConkey agar by Surface-Spread Plate technique and plates were incubated at 35°C for overnight. Aerobic colony count (ACC) was determined by counting the colonies on nutrient agar plates. The identification of the organisms was determined by their morphology, culture characteristics and biochemical profile. The ACC range of salad samples was found to be 1.0×10^{3} cfu/g to 5.8×10^{8} cfu/g. Among these, 22% samples showed unsatisfactory level of ACC and 20% were at borderline. The highest ACC (cfu/g) was found in dry vegetables salads (5.8×10^{8}) and least microbial loads (1.0×10^{3}) were observed in vinegar-containing vegetable salads. Among Gram-negative rods *Klebsiella* spp. (16%) were isolated most frequently followed by *Enterobacter* spp. (11%). Whereas among Gram-positive cocci, *Enterococcus* spp. (13%) was foremost followed by *Staphylococcus aureus* (7.5%). This study revealed the potential hazard of RTE salads and it is the need of the hour to perform a surveillance study at national scale.

4.9 Effect of Excessive Environmental Fluoride on Thyroid Morphology and Function in Children- A Review

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Besides Iodine, there are many micronutrients like Se, F, Fe etc which effect Thyroid glands. Among them an important micronutrient is Fluoride, A halogen, more reactive & electronegative than Iodine & an unavoidable mineral, when in excess in earth crust, air & water of certain regions in the world. Its excessive consumption in food & water (> 1.5 ppm) disturbs the Thyroid Hormone Metabolism, As It is more electronegative than iodine, it easily displaces iodine from the body, thereby affects the functioning of thyroid gland. Fluoride has been known to have shown gross as well as biochemical changes within the body of an individual which cause deranged thyroid hormonal level with in the body. The production of thyroid hormones is regulated by a negative feedback mechanism, i.e., when the pituitary gland senses a drop in FT_3 levels in circulation, it releases more TSH to stimulate the thyroid gland which in turn accelerates the production of the thyroid hormone T_4. The major source of circulating T_3 is from peripheral deiodination of T_4 and not from thyroid secretion. The enzymes which catalyze deiodination are called iodothyronine deiodinases and fluoride is known to interfere with the activity of the deiodinases thereby causing Degenerative Changes in Central Nervous System,impairment of brain function & abnormal development in Children which appear in the form of Dental & Skeletal Fluorosis .

Endemic Fluoride areas are found in India,Sri Lanka,China,Spain,Itly,West Indies,& some part of USA. In Pakistan there are also certain endemic Fluorosis areas where excessive Fluoride in the Environment is affecting Thyroid gland function & becoming reason of mottled teeth, knock knee, bow legs,deaf mutism & low IQ. There is a need not only to mark these areas but their effects should also be reduced.We are underway in finding this problem in an endemic fluorosis area in Manga Mandi near Lahore.

4.10 Evaluation Of Biochar And Compost Antifungal Potential Against Grey Mold Disease

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Biochar produced from corn cob and food waste compost were selected to find out their antifungal properties against *Botrytis cinerea* Pers. Ex Fr. causal agent of grey mold disease. Extracts of biochar and compost were prepared in methanol and distilled water and their different concentrations were tested *in-vitro* against *B. cinerea.* All the applied concentration of methanolic extract of Biochar viz. 0.1%, 0.2%, 0.3% effectively inhibited the growth of test fungus. However, 0.2% conc. of methanolic biochar extract more effectively retarded the dry biomass of test fungus upto 55%. Methanol extracts of compost and distilled water extracts of both biochar and compost were also found effective in suppressing the growth of *B. cinerea.* Methanolic extract of biochar was further subjected for fractional guided bioassays. Different organic fractions from biochar extract were isolated viz. n-hexane, chloroform, ethyl acetate and n-butanol in increasing polarity order. These isolated fractions were further serially diluted to check their MIC along with synthetic fungicide Metalaxyl+ Mancozeb and two controls; Dimethyl sulphoxide (DMSO) and distilled water. The MIC was recorded for each fraction in interval of 24, 48 and 72 hours. The ethyl acetate and fungicide were found to be most effective with MIC of 0.0097 mg mL^{-1} followed by n-butanol fraction. Whereas n-hexane and chloroform fractions were found ineffective.

4.11 Interactive Effects of Salinity and Boron on the Growth, Physiological, Antioxidative Enzymes and Yield Responses of Wheat (*Triticum aestivum* L.)

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A pot study was conducted to evaluate the effect of combined stresses of B toxicity and salinity on the growth, yield, physiological and biochemical processes of wheat. This pot study comprised of twelve treatments including four levels of B (control, 2.5, 5 and 7.5 mg kg^{-1}) and three levels of salinity (control, 100 and 200 mM NaCl). Two wheat genotypes SARC-I (salt-tolerant) and MH-97 (salt-sensitive) were used in present pot study. The results showed that at lower level of B i.e. 2.5 mg kg^{-1}, the growth, yield and physiological attributes of wheat was improved at both levels of salinity. While the high B and salinity together resulted in higher reduction in growth and yield of wheat than their individual effects. However the reduction in growth and yield of wheat by combined salinity and high B (5 and 7.5 mg kg^{-1}) was less than the sum of reduction caused by individual salinity and high B. The tolerant and sensitive wheat genotypes showed variations in salinity and B stress tolerance, Na^+ and K^+ accumulation, antioxidant enzymes (SOD and CAT), growth and physiological responses. The salt-tolerant genotype produced better yield when exposed to B toxicity as well as combined salinity and B stresses than salt sensitive genotype. The activity of SOD and CAT increased with increasing salinity and B stresses either alone or in combination and was found higher in tolerant genotypes compared to sensitive genotypes. The leaf concentration of Na^+, Cl^- and B was higher while that of K^+ was lower in sensitive genotype than tolerant genotype. The leaf B concentration was increased at lower rate of applied B (control and 2.5 mg kg^{-1}) in soil with increasing salinity and decreased at high soil B application rate. The leaf Cl^- concentration increased with increasing salinity while decreased with increasing B in soil. Under salinity stress, B application at 2.5 mg kg^{-1} resulted in better growth, yield and physiological features, higher K, lower Na^+, B and activities of SOD and CAT representing reduction in negative effects of salinity, however, higher B levels further reduced growth and yield of wheat.

4.12 Probiotics: A Potential Therapeutic Tool Against Nonalcoholic Fatty Liver Disease

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Probiotics are live microbes able to modulate the intestinal microflora and enhance body health. At birth, the gastrointestinal tract is a sterile environment. Within a few months after birth, a relatively stable microbial population is established. Nonalcoholic fatty liver disease (NAFLD) is characterized by aberrant lipid storage in hepatocytes (hepatic steatosis) and inflammatory progression to nonalcoholic steatohepatitis. The majority of patients with nonalcoholic fatty liver disease (NAFLD) are either overweight or obese, and there is convincing evidence that NAFLD is a component of the metabolic syndrome. Currently, it is the most common liver disease worldwide, both in adults and in children. Scientific evidences showed that intrahepatic lipid accumulation does not always derive from obesity. Gut microbiota has been considered as a regulator of energy homeostasis and ectopic fat deposition, suggesting its implications in metabolic diseases. The molecular mechanism of probiotics is not completely understood but many of their effects have proved to be beneficial in NAFLD. These include i) modulation of the intestinal microbiota, ii) an antibacterial substance production, iii) an improved epithelial barrier function, iv) reduced intestinal inflammation. Given the close anatomical and functional correlation between the bowel and the liver, and the immune-regulatory effects elicited by probiotics, the aim of this review is to summarize today's knowledge about probiotics in NAFLD, focusing in particular on their molecular and biochemical mechanisms, as well as highlighting their efficacy as an emerging therapeutic strategy to treat this emerging ailment.

4.13 Signature role of miRNA in cell and therapeutic value in the treatment of Acute Myeloid Leukemia

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Cancer is a rapidly spread disease worldwide and Leukaemia is the 8th most common cause of cancer death, with around 53,800 deaths in 2012 (3% of the total) in Europe. MicroRNAs (miRNAs) genes are large family of highly conserved non-coding RNAs of 19-25 nucleotides in length that regulate gene expression by targeting protein-coding mRNAs, related to development, differentiation, tumor suppression (miRNA family, let-7b and let-7c), apoptosis and proliferation. The biogenesis of miRNAs begins in the nucleus and is completed in the cytoplasm, the human genome encodes more than 1,000 unique mature miRNAs, each of which regulate hundreds, may to regulate approximately 60% of all human genes. Any abnormality in the MicroRNAs results in to the cancer. . MiR-221 and miR-222 are examples of miRs that act as oncogene targeting and inhibiting the expression of the tumor suppressor gene, p27Kip. The miR15 and miR16 are located at chromosome 13q14, a deleted region found in more than half of B cell the lead to genesis of lymphocytic leukemia especially in CD5 + lymphocytes. MiR-15 and miR-16 have been shown to target the BCL2 oncogene, leading to its down-regulation and, consequently, resulting in apoptosis in leukemic cells. Recently, miRNAs have been shown to play a role as both targets and effectors in gene hypermethylation and silencing in malignant cells. The enforced expression of miR-29b in acute myeloid leukemia cells resulted in marked reduction of the expression of DNA methyltransferases DNMT1, DNMT3A, and DNMT3B by targeting its regulator Sp1 at both RNA and protein levels. This in turn led to decrease in global DNA methylation and re-expression of p15 ^{INK4b} and ESR1 via promoter DNA hypomethylation. The small noncoding RNAs, named microRNAs (miRNA) regulate gene expression at post-transcriptional level, for the most part by binding through partial sequence homology to the 3` untranslated region of target mRNAs and causing block of translation and/or mRNA degradation. The potential of using these small RNA s as therapeutic targets opens up new opportunities for leukemia therapy by either inhibiting or augmenting their activity.

4.14 Evaluation of phytochemical, antioxidant, hemolytic and stabilization of sunflower oil by *Amomum sabulatum* (Large cardamom) leaves extracts

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Herbal drugs are frequently considered to be less toxic and also free from side effects, than synthetic ones. Hence, the present study was designed to evaluate phytochemical, antioxidant and hemolytic activity of *Amomum sabulatum* (Large cardamom) leaves. In present research work, the leaves were extracted by maceration with increasing polarity based solvents i.e. n-hexane, chloroform, ethyl acetate, n-butanol and methanol. The qualitative analysis of *Amomum sabulatum* leaves was carried out for phytochemicals. Alkaloids, flavonoids, saponins, , cardiac glycosides, phlobatannins, saponins tannins were found present and steroids, terpenoids were absent. TPC and TFC assessment was also carried out the amount was found highest in methanol and lowest in n-hexane. Antioxidant potential was measured by DPPH free radical scavenging and linoleic acid oxidation assays. BHT was used as a positive control in percentage scavenging assay. The percentage inhibition by linoleic acid oxidation was measured for antioxidant studies. The reducing power of extracts was also evaluated. The extracts will also be used for the stabilization of sunflower oil. Finally hemolytic activity of *Amomum sabulatum* extracts was evaluated.

4.15 Drought induced alterations in growth and *in vitro* regeneration of bread wheat (*Triticum aestivum* L.)

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Responses of the wheat commercial cultivar 'Faisalabad-2008' to various concentrations of 2,4-D and the effect of water stress on callus induction and regeneration efficiency were evaluated. For the assessment of responses to water stress, growing morphogenic calli of 7, 14 and 21 days after culture were exposed to different concentrations of water stress. For induction of stress wheat calli were exposed to different concentrations of PEG 6000 (0, 4, 8, 12 and 16% (w/v) and mannitol (0, 0.5, 1, 1.5 and 2% (w/v). The effect of these stresses on shoot and root length, relative water content and electrolyte leakage were also determined. The results indicated that maximum regeneration was observed at 5 mg/L of 2,4-D, increase or decrease in 2,4-D concentration resulted a decrease in regeneration efficiency. Maximum regeneration was observed from 21 days old calli. Significant decreases in the number of shoots regenerated per calli; shoot and root length and relative water contents occurred under both stresses with the highest reduction under mannitol-induced osmotic stress. Electrolyte leakage increased at higher PEG and Mannitol concentrations due to membrane damage. Furthermore, both PEG and mannitol induced drought stress efficiently but effects of mannitol were more severe. Only explants competent to tolerate selective agent survived in the long run and were selected. Selected cells were subsequently regenerated into plantlets due to their 'totipotency'. The protocol proposed here can effectively be used for in vitro screening and improvement of wheat for drought tolerance.

4.16 Relation of Weight gain and Dyslipdemia in Teenagers by Excess Consumption of Fast Food and comparasion of Physical Activities

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Fast foods are frequently linked to the epidemic of obesity. Childhood obesity is an escalating issue that is detrimental as it affects serum lipid profile and Body Mass Index (BMI). Obesity in childhood increases the risk of heart diseases due to which mortality rate accelerates to peak. Many of today's children routinely consume more calories than they expend in physical activity. The following study will certainly look into effect on Serum Lipid Profile and Body Mass Index (BMI) due to fast foods consumption and less physical activity in children. At random, 24 children (both gender, ages 13-19 yrs) will be divided directly into two groups. One group will follow frequently fast foods habitual diet (4-5 times in a week) and second group will take in normal habitual diet for 40 days in 3 intervals (base line, 20^{th} day and 40^{th} day). General physical examination, complete food history, Body Mass Index (BMI), waist circumference will be checked. Blood samples regarding lipid profile will be analyzed. Best statistical check-up will be employed.

4.17 Effect of Rhizobacterial Mediated Auxin on Growth Promotion of Wheat and Mung Bean Plants

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Pakistan is an agricultural country and 90% of its economy depends on agriculture. Thus, better growth and yield of crops is necessary for food and economic progress. Rhizobacteria increase the growth and productivity of plant. The best alternate might be PGPRs which are potential tools for sustainable agriculture and trends for the future. The objective of this study was to explore the indigenous beneficial rhizobacteria for growth promotion of wheat and Mung bean plants. IAA (auxin) is the most quantitatively important phytohormone produced by PGPR. In order to isolate the rhizospheric bacteria different agricultural soil samples from southern punjab area were collected. The isolates were screened on the basis of IAA production, 40 strains (WUMF1-WUMF40) were selected morphologically and biochemically characterized and its observed that 58.9% (WUMF-2, WUMF-8, WUMF-12, WUMF-16, WUMF-19 WUMF 25, WUMF32, WUMF-35, WUMF-38, WUMF-40) were gram positive and negative rods respectively. Gram negatively rods were found to contain citrase and oxidase enzyme. Auxin production potential checked in different environmental conditions at variable PH and temperature and maximum Auxin production was calculated, all the strains were applied on *Triticum estivum* and *Vigna radiate* plants and observed their effect on plant growth promotion in laboratory condition. Significant increase in root length, shoot length, fresh weight and dry weight of strain (WUMF-20, WUMF-35and WUMF38) was observed. The evaluation of rhizobacterial strains for PGP characteristics exposed that (WUMF-20, WUMF-35and WUMF38) strain had maximum PGP characteristics (ACC deaminase production, phosphate solubilization, siderophore, protease, pectinase and chitinase) that may involve in plant growth promotion. This study may be helpful in comparing effect of PGPR on plant growth promotion and selecting the effective natives candidates in agricultur for further research and field implementation.

4.18 Comparative effects of garlic (*Allium sativum*) aqueous and ether extracts on alloxan-induced diabetic rats

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Among plant remedies, effectiveness of black cumin (*Nigella sativa*) in reducing diabetic complications has well established. The present study was therefore designed to compare the effects of garlic (*Allium sativum*) extract to black cumin (NS) in alloxan induced diabetic rat. Diabetes was induced in Albino rats weighing 240-260g byalloxan monohydrate (150 mg/kg body weight). Rats were randomly allocated into six experimental groups; GI: normal control rats (receiving isotonic NaCl); GII: diabetic rats (receiving alloxan administered; 150mg/Kg); GIII: diabetic rats treated with garlic aqueous extract (AGE; 150mg/Kg); GIV: diabetic rats treated with garlic ether extracts (EGE; 150mg/Kg); GV: diabetic rats treated with insulin (6 to 8 IU), and GVI: diabetic rats treated with *Nigella sativa* oil (NSO; 150mg/Kg). Garlic aqueous and ether extracts wereadministered orally to diabetic rats for two weeks after which the blood samples were collected to determine the fasting blood level and the serum was separated to analyze the lipid profile. The diabetic rats (GII) were compared to normal control rats (GI), while all other treatment groups (GIII, GIV, GV, and GVI) were compared to diabetic rats (GII). Body weight of rats was calculated before and after the administration of garlic extracts. Administration of alloxan has significantly reduced (-20%, $p<0.001$) the mean body weight of diabetic (GII) rats. Both, the garlic aqueous and ether extracts (GIII and GIV) showed no significant effect (-2% and 1% respectively, $p>0.05$) in increasing the body weight of diabetic rats when compared with insulin treated (23%, $p<0.001$) and NSO treated (48%, $p<0.001$). NSO treatment showed the maximum increase in body weight of diabetic rats. While comparing the relative efficacy of garlic aqueous and ether extracts in lowering the mean blood glucose level of diabetic rats (324%, $p<0.001$), the garlic aqueous extract (AGE) has showed the better activity in decreasing the blood glucose level (-56%, $p<0.05$). Among all the treatment groups, NSO treatment to rats showed the less activity (-27%, $p<0.001$). Obviously, the insulin treatment has showed the significant effects in normalizing the mean body weight and blood glucose level of diabetic rats. Analysis of lipid profile has showed that both the garlic extracts proved better remedy, however, the garlic ether extract (EGE) was found very effective in normalizing all the lipid contents; total cholesterol (TC), triglycerides cholesterol (TG-C), high density lipoprotien (HDL-C), low density lipoprotien (LDL-C), and very low density lipoprotien (VLDL-C). Treatments with insulin and NSO, both showed notable reduction of HDL and elevation of LDL contents in diabetic rats that were not comparable to the diabetic control and/or the normal control values. From the findings it can be concluded that garlic extracts have showed the significant hypoglycaemic and lipid normalizing activity and may potentiate the hypoglycaemic effect of antidiabetic drugs If taken combined with conventional drugs.

4.19 Comparative Study Of Exogenous Application Of Plant Growth Regulators Against Urea Fertilizer On The Biochemical And Phenolic Antioxidants Of Mint Leaves

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Availability of freshly grown leafy vegetables available at cheaper cost can assure the end of hunger in developing countries including Pakistan. It also tries to reduce the marginal gap between demand and supply of food among the common people. Therefore, this topic has gained attraction in scientific research to benefit mankind. Present study investigated the effectiveness of exogenously applied plant growth regulators on the biochemical and antioxidant attributes of mint plants grown in normal and urea enriched soil. The selected plant growth regulators included 6-Benzyl amino purine (75 ppm), freshly prepared *Moringa oleifera* leaf extract (1:30 M:L) and humic acid (10%). The foliar spray of these growth regulator was applied three times (@ 160 L/hectare), 1st after 25-30 DAS (days after Sprouting) and 2nd and 3rd spray after two weeks of first spray, respectively. The experiment was also categorized on the basis of soil mode as A (no urea in the soil) and B (urea enriched soil). The mint leaf samples were collected at three different stages i.e. 40, 50 and 60 days respectively and were analyzed for biochemical and antioxidant analysis. Among biochemical parameters, mint leaves showed contents of proline as: 14.59-43.17 ug/g in A and 10.87-37.23 ug/g Fresh Mass (FM), melondialdehyde (MDA) as 2.74-27.15 and 11.36-35.51 ŋg/g FM, total soluble proteins as 3.95-26.31 and 5.47-28.47 ug/g FM in A and B mode, respectively. Meanwhile, the study of chlorophyll and carotenoids revealed the following values; 1.623-7.306 and 1.745-9.284 mg/g FM; and 0.218-0.876 and 0.313-0.893 mg/g FM, in A and B experiment, respectively. Total phenolic contents were found to be 9.28-23.02 in A and 4.54-14.15 mg Gallic acid equivalent (GAE) in B mode respectively. The results of antioxidant assays depicted the values as: 0.822-1.904 and 0.816-1.640 (reducing power at 10 mg/mL) and 0.127-0.441 and 0.153-0.489 (DPPH°: IC_{50} value). Furthermore, a list of phenolic acids was also studied on the basis of HPLC analysis. The results showed that samples treated with plant growth regulators (A and B mode) gave significant results against negative control (no exogenous application) and positive control (Water was applied @160L/Hectare). The overall study concluded that plant growth regulators yielded comparable results as that of urea enriched soil and hence can be preferred over synthetic fertilizers being ecofriendly and economically.

4.20 Compositional difference in Antioxidant and Antibacterial Activity of All Parts of the *Carica Papaya* Using Different Solvents

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Carica papaya is a well known medicinal plant used in the West and Asian countries to cope several diseases. Patients were advised to eat papaya fruit frequently during dengue fever epidemic in Pakistan by physicians. This study was conducted to establish mainly profile of Polyphenols, flavonoids and antioxidant potential of extracts of all major parts of the *C. papaya* with seven major solvents i.e. water, ethanol, methanol, n-butanol, dichloromethane, ethyl acetate, and n-hexane. TPC, TFC, antioxidant and antibacterial potential were determined using different aqueous and organic solvents in addition to the determination of trace element in leaves, pulp and peel of *C. papaya*. Total soluble phenolics and flavonoids were found in promising quantity (≈ 66 mg GAE /g) especially in case of methanol and ethanol extracts. Antioxidant activity using DPPH free radical scavenging assay indicated leaves, bark, roots and pulp extracts showed > 75.0% scavenging potential while leaves and pulp showed 84.9% and 80.9% inhibition of peroxidation, respectively. Reducing power assay showed leaves, pulp and roots extracts active to reduce Fe^{3+} to Fe^{2+} ions. The antibacterial study showed pulp extract is the best to cope infectious action of bacteria.This study was conducted to test the medicinal profile of all parts of *C. papaya* by extracting secondary metabolites with organic and aqueous solvents. Ethanol and methanol both were found to be the best solvents of choice to extract natural products to get maximum medicinal benefits and could be used to medicinal formulation against different infectious diseases.

4.21 Phytochemical Evaluation of *Allium sativum* L. and *Zingiber officinale* L. Rhizome in various organic solvents

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Despite the enormous progress in conventional medicinal system, phyto-medicines are still a common practice for the treatment of diseases. The objective of the present study was the phytochemical screening and determination of antioxidant potency of *Allium sativum* and *Zingiber officinal*e in aqueous, *n*-hexane and chloroform fractions. Total phenolic contents of *Allium sativum* rhizomes were in the range of 29.2-34.5 mg GAE/g, whereas these were 32.4-38.0 mg GAE/g for *Zingiber officinale.* Regarding solubility in solvents, following trend was observed: *n*-hexane > water >chloroform. Total flavonoid contents of *A. sativum* ranged from 0.24 to 0.35 CE mg/g whereas, it was 0.39-0.49 (CE mg/g) in case of *Z. officinale.* Highest flavonoid concentrations were observed in chloroform extracts of *A. sativum*, followed by water and *n*-hexane. However, in case of *Z. officinale*, optimal extraction was measured in aqueous fraction followed by trending as chloroform > *n*- hexane. DPPH radical scavenging activities ranged from 50 to 69% and 68-83% for *Zingiber officinale* and *Allium sativum* respectively. The least scavenging activity was exhibited by water extract (50%) for both plants and DPPH scavenging activity in descending order was: *n*-hexane > chloroform> water. This study endorses further research directed at isolation, purification and characterization of active components that may reveal new avenues in functional foods.

4.22 Application of metabolomics in characterizing the milk

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Bovine milk is the most widely consumed beverage in the world. Milk of bovine origin is both consumed fresh and processed into a variety of dairy products including cheese, fermented milk products, and infant formula. It is a nutritionally rich, chemically complex biofluid consisting of hundreds of different macro- and micronutrients.The nutritional quality and processing capabilities of bovine milk is closely associated to milk composition.While the chemical composition of cow's milk has been studied for many decades, much of this information is fragmentary, outdated and scattered in hundreds of specialized books and journals. In an effort to both consolidate and substantially update this information, we have applied a combination of modern, quantitative metabolomics techniques along with state-of-the-art, computer-aided literature mining techniques to obtain the most comprehensive and up-to-date characterization of the chemical constituents in cow's milk. In particular, using nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC-MS), direct flow injection mass spectrometry (DFI-MS/MS), inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC), we were able to quantify and validate more than 250 bovine milk metabolites from a variety of commercial milk samples. The computer-aided literature review led to the identification of another 150 milk metabolites. Compared to other previously published bovine milk composition studies, this represents a X-fold increase in metabolite coverage and a Y-fold increase in available concentration data. All of the identified bovine milk metabolites along with their concentrations, structures, related literature reference, and links to their associated diseases have been made freely available through a web-accessible database called MilkDB (http://www.cowmetdb.ca/).

POSTER PRESENTATIONS

TRACK 4

4.1-P Phytochemicals, antioxidants and haemolytic studies of *Calendula officinalis* (Pot marigold) leaves extracts

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In the current study of *Calendula officinalis* belong to the family Asteraceae, leaves were analyzed for Phytochemical, antioxidant, and cytotoxicity studies. The leaves of *Calendula officinalis* were mcerated absolute solvents *n*-hexane, chloroform, ethyl acetate, *n*-butanol and 80% methanol. The qualitative investigation of phytochemical study such as alkaloids, flavonoids, saponins, tannins, steroids and terpenoids were studied. The full amount of phenolics and flavonoids content were evaluated by means of spectroscopic method. The plant leaves enclosed considerable values of total phenolic contents (17.12-282.22 GAE mg/100 g of dry extract) and total flavonoid contents (7.26-199.6 CE mg/ 100 g of dry extract). DPPH percentage inhibition and percentage inhibition of linoleic acid peroxidation were studied. The haemolytic effect of the plant leaves extracts was found in range of 0.84-5.63%.

4.2-P Comparison of Blood Analytes in Marasmus and Kwsashiorkor Affected Children

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Malnutrition is globally the most important risk factor for illness and death. Micronutrient deficiencies are significant health problems among infants and young children in rural areas of Multan, Pakistan. Marasmus and Kwashiorkor are major health problem contributing to the burden of disease in developing countries. This study aimed to compare the deficiencies of blood analytes in Marasmus and Kwashiorkor affected children. We had collected the data of 300 children (100 control, 100 Kwashiorkor, 100 Marsmus) within the age range of 4-10 years from Children Complex Hospital, Multan, Pakistan. Result showed that serum level of sodium and creatinin were high whereas, the levels of potassium and calcium were lower in both Marasmus and Kwashiorkor than control subjects. The levels of leukocytes, platelets and hemoglobin were decreased in both diseases. Erythrocytes level was decreased in Marasmus while increased in Kwashiorkor. Findings of the study highlight inadequate feeding practices, low nutritional quality of meals and high prevalence of stunting. Feasible strategies are needed to address the dietary inadequacies and chronic malnutrition of rural infants.

4.3–P Frequency of Thyroid Disorders in Ischemic Heart Disease Patients: A Single Centre Experience in Pakistan

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Pakistani population has one of the highest risks of cardiovascular diseases (CVD) in the world. The major risk factors are tobacco use, hypertension, obesity, diabetes and unhealthy diet. Being iodine deficient country an additional risk factor may be thyroid disorders (TD) but its role in etiology of CVD is yet to be determined in Pakistani population. We determined frequency TD in Ischemic heart disease (IHD) patients (n=298) referred to CENUM, Mayo Hospital during calendar year 2010 to 2013. Serum free thyroxin (FT_4) and thyroid stimulating hormone (TSH) were detected by radioimmunoassay (RIA) techniques. The age range of patients was 20 to 85 year. About one-fourth of patients (n=75; 25.2%) had abnormal serum TSH and 41 (13.8%) had severe TD with almost same frequency of hyperthyroidism (n=19; 6.4%) and hypothyroidism (n=22; 7.4%). The ratio of male to female was same (1:3) in those with and without TD and there was no gender based difference in frequency of TD (13.3% versus 13.9%; p= 0.993). Among studied patients 52 (21.1%) had concurrent diabetes (DM) but incidence of TD was not increased due to presence or absence of DM (11.5% versus 14.2%; p=0.887). A high frequency of TD was found in patients ≤ 50 year of age (20/136; 14.7%) as compared to those above this limit (11/162: 6.8%) but the difference was not significant (p=0.079). Thus a high frequency of severe TD that was independent of patient's gender, age and concurrent presence of DM necessitates exploring role of TD as one of the risk factor for CVD in local population.

4.4-P Phytochemical and antioxidant studies of *Persea americana* (Avocado) fruit extracts

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Medicinal plants are a rich source of new drugs. Many of the modern medicines are produced indirectly from medicinal plants. These medicinal plants contain such ingredients which can be used to treat diseases and involved in drug synthesis. *Persea americana* (avocado) was analyzed for its phytochemical and antioxidant activity. The material of plant fruit were extracted with n-hexane, chloroform, acetone and 80% methanol. The qualitative analysis was carried out for phytochemicals like alkaloids, flavonoids, steroids, saponins and tannins. The total flavonoids and total phenolic content was analyzed by spectroscopic techniques. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity along with percentage inhibition of peroxidation was carried out and results were analyzed statistically.

4.5-P Influence Of The Gamma Radiation Dose On Micro Flora Of Deboned Broiler Chicken Meat

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Chicken meat *Gallus gallus domesticus* is a very common food in world due to its low caloric density and high biological value, which makes it perfect for weight management diets. Irradiation is the significant way of sterilization of food from microbes. This study was conducted to determine the microbial load on Broiler chicken and their elimination by gamma radiation and enhance the shelf life of Broiler chicken. Broiler chicken samples were irradiated at 0.5, 1 and 1.5kGy doses by gamma radiation source Co60. Then samples were tested to evaluate the presence of microbes *Campylobacter jejuni, Escherichia coli, Staphylococcus aureus, Salmonella* spp., on broiler chicken by total viable count, *Staphylococcus* count, coliform count, yeast and mold count and hemolytic bacterial count. In addition to this, in these samples microbes were also checked for endospore count, antibiotic resistance for gentamicin, ampicillin and vancomycin and acid resistance at pH 2 and 3. After radiation there was a significant reduction in total viable count in Broiler chicken meat. Coliform count and yeast and mold count were also decreased to a safe level for human consumption. Antibiotic and acid resistant bacteria were also reduced at this radiation. Hence, gamma radiation of dose 1.5KGy is highly recommended to make Broiler chicken meat safe for human consumption.

4.6-P Purification and production of l-asparaginase from *E.coli*

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Enzymes are biological molecules, selective catalyst and responsible for the interconversions that sustain life. Asparaginase is an enzyme that is responsible for the hydrolysis of asparagine to ammonia and aspartic acid. L-asparaginase is an anticancer agent and used for sharp lymphoblastic leukemia chemotherapy. In bacteria, animal and plant tissues, this enzyme is present but absent in the humans. The present study was focused on the production and purification of L-asparaginase from *E.coli*. Crude enzyme was subjected to purification. To purify the enzyme various techniques were used such as dialysis and ion exchange chromatography. Desalted sample showed activity and specific activity of 43.62U/mL and 4.622Units/mg respectively. In ion exchange chromatography, 10^{th} fraction had maximum activity of 37.92U/mL. After purification enzyme activity was analyzed at various parameters such as pH and temperature. The enzyme activity was found to be maximum at pH 9 and temperature 50°C. The values of V_{max} and K_m were 3U/mL and 133.3mM respectively. Activation of enzyme was observed at 1000µg/mL by using MgCl2 as activator. Inhibition of enzyme was observed at 4µg/mL by using EDTA as inhibitor.

4.7-P Minimization Of Epiphytic Microbial Flora On Garlic To Enhance The Shelf Life Employing Gamma Radiations

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Garlic is a well-known spice used in many Asian, African and European countries. Its significant chemical composition makes it medically important as garlic exhibited antimicrobial, antioxidant, anticarcenogenic, antidiabetic activities. Microbial spoilage of garlic is the prime reason of postharvest spoilage every year. In the present study, gamma irradiation was used to reduce the pathogenic surface micro flora of *Allium sativum* hence prolonging the storage life. "Desi" variety of garlic bulbs was selected for gamma radiation treatment. The garlic samples were irradiated at different doses of 0.25, 0.50 and 0.75kGy. Analysis of Organoleptic qualities and native microbial load was carried out for both irradiated and non-irradiated garlic. The results revealed that gamma irradiation significantly reduced the micro flora without causing harm to the organoleptic qualities of garlic at 0.5kGy and enhanced shelf life for 1 month as compare to non-irradiated garlic.

4.8-P An evaluation of phytochemicals, antioxidants and cytotoxicity of *Solanum tuberosum leaves* extracts

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In the present study of *Solanum tuberosum* belonging to the family *Solanaceae,* leaves were evaluated for phytochemical and antioxidant studies. The leaves were extracted by maceration with n-hexane, ethyl acetate, chloroform and 80% methanol. The qualitative determination of phytochemicals such as alkaloids, flavonoids, saponins, tannins, steroids and terpenoids were analyzed. The total phenolics and flavonoids content were analyzed by spectroscopic technique. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and linoleic acid oxidation assays were carried out. The percentage inhibition by DPPH and percentage inhibition by linoleic acid oxidation was also analysed. The fractions of plant extract were assayed against human red blood cells (RBCs) and the percentage lyses was found to be in the range of 2.04 to 6.23 %. Results were analyzed statistically

4.9-P Nutritional Assessment Of Different Date Varieties And Their Parts

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Back ground: Date palm (*Phoenix dactylifera* L.) is one of the oldest food crops of mankind's food being cultivated mostly in Arab gulf areas. Ajwa date can be distinguished from other date varieties due to higher nutritional properties and also being favorite date of Holy prophet (PBUH). However, there is a little work on comparative analysis of ajwa flesh and pits and also with locally available date cultivars. Proximate composition, mineral analysis and sugar profile of date parts (flesh and pits) of all varieties were carried out to access comparative nutritional profile. Correlation between proximate variables of all date varieties and within different minerals was analyzed. Results indicated ajwa flesh as richest source of ash (3.22 %), moisture (22.8 %) glucose (54.5%), fructose (52.03%), maltose (22.5%), and galactose (12 .2%) contents while ajwa pits have highest amounts of crude fat (7.8%), crude fiber (51%), TDF (53.9%), IDF (34.6), and SDF (19.5%). Highly significant positive correlation of crude protein with crude fat and crude fiber was observed whereas crude fat and crude fiber also exhibited positive correlation. Na exhibited highly significant negative correlation while Zn and Ca has positive correlation with all other minerals. The work classifies variability among flesh and pits of ajwa date and local date varieties for their nutritional characteristics emphasizing their use in food industry as functional ingredient in food products and supplements.

4.10-P Influence Of Silicon Application on Chickpea (*Cicer arietinum* L.)

Under Salinity Stress

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Chickpea is one of the best essential grains leguminous plants more over fulfill requirements of food for animals and human was reported to lower yield arid and semi-arid areas during salt stress because of Na^+ and Cl^- ions accumulation that leads to reduce growth during saline environment. But Silicon is the element that shows increase resistance against salinity. The Silicon provides resistance to plant to avoid salinity by alleviating the adverse impact of the stress. The present research was conducted to evaluate the silicon application to increase tolerance in chickpea (*Cicer arietinum.* L) against salinity stress by determining the changes in germination as well as other physiological and biochemical parameters at vegetative, reproductive and harvesting stage. Two experiments were conducted Pot and Petri plates. Pots were arranged in randomized complete block design with set of nine treatment each containing three replicates. The treatments were control, 50 mM NaCl, 100 mM NaCl, 50 ppm Si, 100 ppm Si, 50 mM NaCl + 50 ppm Si, 50 mM NaCl + 100 ppm Si, 100mM NaCl + 50 ppm Si & 100 mM NaCl + 100 ppm Si. Sodium Chloride under both concentrations (50mM &100mM NaCl) was responsible for significant decrease in germination parameters that is germination percentage, seedling vigor index, shoot length, root length , fresh and dry weight of seedlings, Tolerance index. During vegetative and reproductive stage both concentration of sodium chloride caused increase in proline content, chlorophyll content, osmotic potential, sugar content while decrease in relative water content, membrane stability index and protein content. Whereas silicon application under salinity stress provide significant improvement in different parameters which were negatively influenced by salinity stress. So it can be concluded that application of Silicon help in mitigating the adverse effects of salt stress on growth, physiology and yield of chickpea.

4.11-P Alteration in the antioxidant activity of *Brassica rapa* applying different plant extracts

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Seed priming has been used effectively for the imporvement of propagation and growth of most vegtables and field crops. It increases the germination rate, total germination percentage and germination uniformity. Plant growth regulators (PGRs), are small organic molecules that act inside the plant cells and increase the growth and development of plants. Vegetables are essential crops for extra source of human nutritional necessities. Plant leaf extracts are used as growth regulater for various crops due to their active phytochemicals, easy preparation, low cost and environmentally friendly nature. The present study has been desinged to evalute different plant extracs as priming agents on seed germination, seedling growth, antioxidant activity and chlrophyl content of turnip. *The antioxidant activities of Turnip extracts will be assessed using different antioxidant assays such as, estimation of total phenolic contents (TPC),* reducing power and DPPH radical scavenging assay. *The proximate composition will be assessed by AOAC methods. The data will be statistically analyzed by using* ANOVA test.

4.12-P Antioxidant, phytochemical and haemolytic studies of *Daucus carota* (Carrot) leaves extracts

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Daucus carota have a great medicinal importance. In view of its tremendous medicinal properties, leaves of *Daucus carota* plant have been selected in the present research work for evaluation of phytochemical, antioxidant and haemolytic studies. The leaves were extracted by maceration with increasing polarity based solvents i.e. n-hexane, chloroform, ethyl acetate and 80% methanol. The qualitative analysis was carried out for phytochemicals like alkaloids, flavonoids, saponins, steroids, terpenoids, cardiac glycoside, phlobatannins, saponins and tannin. TPC and TFC assessment was also carried out. Antioxidant potential was measured by DPPH free radical scavenging and linoleic acid oxidation assays. The IC_{50} and percentage inhibition by linoleic acid oxidation was measured for antioxidant studies. The percentage inhibition by linoleic acid oxidation and inhibitory concentration (IC_{50}) was obtained in the range of 34.56 to 91.6 and 99.92 to 83.79 respectively. The reducing power of dry leave powder and extracts was evaluated. Finally haemolytic activity of *Daucus carota* leaves powder and extracts was evaluated and % lysis was obtained from 2.63 to 7.28 % range.

4.13-P An ecotoxicological Study of House Crow in Southern Punjab Pakistan

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A twenty House Crow (*Corvus splendens*) were collected from five districts Rajanpur, Dera Ghazi Khan, Muzaffar Garh, Khanewal and Vehari of Punjab Pakistan. After analysis of bird's samples, it was found, the mean concentrations of copper, cadmium and zinc in liver samples were significantly higher as compared to kidney samples. A highly non-significant value of zinc was observed in liver samples (P=0.175) while in kidney it's significant is (P=0.040). There was no significant difference was found in copper liver samples (0.244) and kidney (0.236). The cadmium concentration in liver samples (0.162) and in kidney is (0.057). There was no significant difference in mean values of metals at each study site in kidney and liver samples. With growing industrial rate in urban areas of Pakistan, the heavy metal toxicity is a major problem that concern with lethal health effects, hence a study was devised to ascertain the level of certain heavy metals including zinc, copper and cadmium in samples of an urban dweller bird species that is House Crow (*Corvus splendens*) The higher values of these metals found in birds will help ultimately aiming the life of fellow humans.

4.14-P Effect of Soil Salinity and Boron Toxicity on the Carbohydrate Partitioning, Growth and Ionic Composition of Two Australian Wheat Varieties

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Research work on the complications of excessive soil boron (B) has augmented noticeably in the current era, but the interactions effects of salinity and toxic B on growth, physiological and biochemical responses of crop plants still need detailed investigations. In this regard, a glasshouse experiment was carried out at Murdoch University, Perth-Australia to investigate the influence of soil salinity and toxic B on carbohydrate partitioning, growth and ionic composition of two Australian wheat varieties (differing in salinity tolerance). The result showed that salt-tolerant Halberd variety accumulated more Na^+, B and Cl^- in their leaf sheath and kept the leaf blades free of these toxic ions than sensitive variety Westonia. Water soluble carbohydrates (i.e., glucose, sucrose, fructose and fructans) concentration increased in response to individual as well as combined constrains of soil salinity and toxic B in leaf blade of both tolerant and sensitive varieties but the increase was higher in tolerant variety as compared to the sensitive one. The concentration of WSCs in leaf sheath of salt tolerant wheat variety was increased in response to stress conditions but those were remained low in salt sensitive one.

4.15-P Antimicrobial, Antioxidant and haemolytic studies by *Citrus limon* (Lemon) leaves essential oils

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The present study has been designed to evaluate the antioxidant, antimicrobial and haemolytic potential of *Citrus limon* leaves. The essential oils and n-hexane extract of *Citrus limon* leaves were analyzed by Gas Chromatography/ Mass Spectrometer which revealed the presence of chemical compounds in essential oils and in n-hexane extract. Both examined essential oils and n-hexane extract of plant leaves showed significant antimicrobial activity. The percentage lysis of red blood cells (RBCs) as analyzed by haemolytic studies was found of the plant essential oils (2.46%) and n-hexane extract (5.75%). Leaf essential oils and n-hexane extract also exhibited a good antioxidant potential when measured using DPPH radical scavenging assay by percentage inhibition. The antioxidant activity of plant essential oils and n-hexane extract was also studied when measured by percentage inhibition in linoleic acid system. The plant essential oils and n-hexane extract showed the values of 81.93% and 50.34%, respectively. When studied by *beta*-carotene-linoleic acid assay, the plant essential oils showed greater antioxidant activity then n-hexane extract. The plant essential oils and n-hexane extract also exhibited good antimicrobial potential against selected fungal and bacterial strains. Considering these results, *Citrus limon* leaves could be used as a source for the exploration of new antimicrobial, antioxidant agents, functional food and nutraceutical applications. 5.63%.

4.16-P Phytochemical, antioxidant and cytotoxicity analysis of *Rosa centifolia* (Gulab*)* leaves extracts

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Plants are very significant from medicinal point of view. Due to natural source plants products are safe. That is why there is a great need of research to estimate plants for their phytochemical and biological properties. In the current study, leaves of *Rosa centifolia*, a member of family rosacea, were analyzed for phytochemical, antioxidant, antimicrobial and cytotoxic studies. There are more than 200 species of Rosa. The leaves were extracted by marceration with n-hexane, chloroform, ethyl acetate and 80% methanol. The qualitative analysis were passed out for phytochemicals like flavonoids, alkaloids, steroids, saponins and tannis. By spectroscopic technique the flavonoids and phenolic content were analyzed. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging action were carried out. Results were examined statically

4.17-P Biochemicals From Sugar Cane Bagasse: A Valueable And Cheap Source

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Bagasse is leftover material after sugarcane crushing. It contains complex polysaccharide base lignocellulosic ingredients, which can be used as low cost carbon source for the growth of microbes. Many microbial metabolites can be produced under several fermentation conditions after pretreatment**.** Now a days, the use of sugarcane bagasse as substitutive substrates of sugars for the production of high value products from enzymes to antibiotics is on rise. It offers several benefits over the traditional carbon sources. Use of the bagasse will bring about the drastic increase in resource productivity needed to make human activity sustainable. Several species of bacteria, yeast and fungi are well known to secrete antibiotics and enzymes on these low cost substrates. The paper will cover the importance of sugarcane bagasse in industrial applications and how its use is environment friendly.

4.18-P Immunological Cross Reactivity Among Salmonella Isolates From Human And Food Animals

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Salmonella enterica causes globally prevailed food born disease, affecting both human and livestock. Salmonella is transferred to the human by animals and their byproducts contaminated with meat, eggs, and dust, and also by contaminated water. In present study the immunological cross reactivity was determined among isolates of Salmonella recovered from human and food animals. Faecal and stool samples were processed and complete identification showed that 60% *Salmonella typhimurium* was isolated from faecal samples of cattle, 60 % from poultry, 60 % from sheep, and 40% from human. Out of total samples, 45% samples were found positive for *Salmonella typhimurium*. Agar gel precipitation test and Serum Neutralization Assay were performed to find out common antigenic moieties among *Salmonella typhimurium* isolates. *S. typhimurium* isolated from cattle showed maximum immunological cross reactivity with antiserum of sheep (1:32) followed by poultry (1:8). Poultry isolates showed maximum relatedness with sheep (1:16). *S. typhimurium* isolated from sheep showed maximum interaction with antiserum of cattle (1:16). Isolates from human showed maximum interaction equally with poultry (1:32) followed by cattle and sheep (1:16). Poultry antigen displayed strong precipitation bands (+ + +) in agar gel precipitation test with sheep and cattle antiserum while medium strength precipitation bands (+ + -) were observed with human antiserum. Cattle antigen displayed strong precipitation bands (+ + +) in with sheep antiserum but faint bands (+ - -) with poultry and no band with human antiserum. Sheep antigen displayed same precipitation bands (+ - -) in with cattle and poultry antiserum. Human antigen displayed medium precipitation bands (+ + -) in with sheep and poultry antiserum while faint precipitation bands (+ - -) were observed with cattle antiserum. Concludingly, the most competent immunological relatedness was found with the *S. typhimurium* isolated from human, ranging from 1:16-1:32 indicating excellent interaction with poultry. This study was helpful to checkout out interspecies cross reactivity of Salmonella isolates and this information may be used for future possible transmission control studies.

4.19-P Vitamin C And E Supplementation Improves Semen Quality Traits Of Retired Male Layer Breeders After Molting

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Forced molting is a common practice used extensively in poultry industry to recycle birds for a second season and to extend their productive life span. The objective of the study was to evaluate the effect of dietary supplementation of protein, probiotics and vitamins (C and E) on semen characteristics and immunohistochemistry of pituitary gland in zinc-induced molted layer breeder males. Two hundred and seventy White Leghorn breeder male birds at 59 weeks of age were included in the study. The birds were forced molted by dietary supplementation of ZnO and were divided randomly into six groups. One group was kept as control and the other five groups were being fed diet supplemented with protein, probiotics, vitamin C, vitamin E and combination of above mentioned supplementations respectively. The birds took about 5 weeks to produce semen after molting. The trial continued for the next 5 weeks during which sampling was performed. Semen volume increased significantly in vitamin C and E treated groups, while percentage of dead sperms decreased in vitamin C group. Immunohistochemistry showed that cell size of FSH gonadotrophs, LH gonadotrophs and lactotrophs were significantly higher in vitamin E and C supplemented group while no significant change was found in somatotrophs cell size and nucleus size. Cumulative effect of vitamins, probiotics and protein was not as good as individual effect of vitamin E and C. This data suggested that vitamins C and E are beneficial in improving the reproductive performance of layer breeder males after molting.

4.20-P The Alarming Indications Of Weight Gain And Dyslipidemia In Teenagers By Excess Consumption Of Fast Foods And Less Physical Activity

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Fast foods are frequently linked to the epidemic of obesity. Childhood obesity is an escalating issue that is detrimental as it affects serum lipid profile and Body Mass Index (BMI). Obesity in childhood increases the risk of heart diseases due to which mortality rate accelerates to peak. Many of today's children routinely consume more calories than they expend in physical activity. The following study will certainly look into effect on Serum Lipid Profile and Body Mass Index (BMI) due to fast foods consumption and less physical activity in children. At random, 24 children (both gender, ages 13-19 yrs) will be divided directly into two groups. One group will follow frequently fast foods habitual diet (4-5 times in a week) and second group will take in normal habitual diet for 40 days in 3 intervals (base line, 20^{th} day and 40^{th} day). General physical examination, complete food history, Body Mass Index (BMI), waist circumference will be checked. Blood samples regarding lipid profile will be analyzed. Best statistical check-up will be employed.

4.21-P Health Benefits of Linoliec Acid

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Conjugated linoleic acid (CLA) belonged to a group of geometric and positional isomers of linoleic acid with conjugated double bond structure which are arranged in different combinations of *cis* and *trans* arrangement. CLA in the diets influence humans as a vital health promoter. Foods that contain high percentage of CLA are meat and milk of ruminants. Positive effects of CLA include anti-diabetic, anti-atherogenic, anticarcinogenic, anti-obesity, immune system improvement, which are isomer specific. CLA also influence insulin resistance, gastrointestinal disorders and gallstone formation Beyond the numerous health benefits associated with Conjugated linoleic acid but still their mechanism of action remains controversial. Therefore, it is important to recognize how different isomers of CLA functions in the body acts to design CLA enriched functional foods from animal origin.

4.22-P Goiter in Female Adolescents with Family History of Thyroid Disease: Is Therapeutic use of Iodized Salt Damaging Their Thyroid Gland?

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Thyroid enlargement (goiter) is a common problem among female adolescence. Mostly this condition during adolescence is physiological and regresses without any treatment. Without knowing their iodine intake status, adolescents presenting with goiter use iodized salt (IS) either on physician recommendation or self-medication to treat this condition in Pakistan. This practice may prove hazardous particularly in those adolescents having family history (FH) of thyroid diseases because of early expression of thyroid autoimmunity (TA). We explored the effect of IS in such female adolescents by determining serum thyroid peroxidase antibodies (TPO-Abs) titer. TPO-Ab is a hallmark of TA and is a best marker of thyroid gland destruction and eventual development of hypothyroidism. During January to June 2014, 157 female adolescents attended CENUM, Mayo Hospital for evaluation of thyroid function tests. Among them 101(64.3%) were presented with goiter. Their mean (±SD) age was 15.3 ± 2.4 years (age range: 9 to 19 years).FH was positive in 29 (28.7%) adolescents. More adolescents with FH use IS as compared to those with no history of thyroid disease (58.6% versus 36.1%; p=0.112). We evaluated TPO-Abs titer in serum of positive FH adolescents. Those using IS (n=17) had more incidence of higher TPO-Abs titer as compared to non user (n=12) (23.5% vs 8.3%;p=0.65). In control group consisting of adolescents with no FH and no use of IS (n=23) incidence of high TPO-Ab was 17.4%. Thus among goitrous adolescents having FH use of IS was more common and they have relatively high incidence of thyroid gland destruction as compared to those not using IS. Further large studies are required to verify these results.

4.23-P Impact of biofilm on the colonization across *Staphylococcus aureus* and *Mycoplasma bovis* under in-vitro conditions

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The poor quality milk production is main public health concern that is associated with mastitis. A total of forty milk samples were collected from clinical and sub clinical bovine mastitis. Biofilm formation property of *Staphylococcus aureus* and *Mycoplasma bovis* helped to understand the pathogenicity of mastitis in bovine. Pure cultures of *Staphylococcus aureus* and *Mycoplasma bovis* were propagated onto the surface of glass coverslip and their biofilm was developed under in-vitro conditions. Individual biofilms were treated with homologous and heterologous culture isolates for the cross colonization potential. The homologous biofilm for *Staph.aureus* and *M.bovis* had resulted into significant increase in growth with mean value of cluster forming units 599.8 / HPF and 558.8/HPF respectively, while the heterologous growth of *Staph.aureus* and *M.bovis* were having mean values of cluster forming units 548.8/HPF and 181.6/HPF respectively. The growth potential of *Staph.aureus* was found equally high irrespective of biofilm source whereas the biofilm of *Staph.aureus* rarely promoted the growth of *M.bovis*. The overall results proved that the initial *M.bovis* infection may have the potential to promote *Staph.aureus* as secondary infections with equal high intensity.

4.24-P Degradationof of AFB_1 in ground nut (*Arachis hypogea*) and tree nuts (*Juglans regia*, *Prunus duclus* and *Pistachio vera*) by gamma irradiation

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The present study was aimed to investigate the effect of gamma irradiation on aflatoxin B_1 (AFB_1) in ground and tree nuts. Samples of nuts were randomly selected from the retail outlets of Faisalabad, Pakistan. The moisture content of the nuts was increased to 10 ± 3 and 16 ± 3%. The nuts were stored for a period of 12 weeks in ventilated cabins.At the end of storage period, all the nut samples were found infected with higher levels of AFB_1 than those set by WHO and IAEA. At high moisture level (16 ± 3%), greater amount of AFB_1 was observed. In ground nut, the highest level of AFB_1 was 158.67 µg/kg.The stored nut samples were exposed to 0, 2, 4, 6 and 8 kGy γ radiation absorbed doses after a storage period of 12 weeks. There was a significant decrease in AFB_1 in all the nut samples after irradiation particularly at higher radiation absorbed dose. The degradation of AFB_1 was found to follow first order rate kinetics. The degradtion rate constants were also calculated. There was a proportional decrease in AFB_1 with increase in irradiation dose in all nut samples at both moisture levels.

4.25-P Correlation Of Total Protein Content And Catalase Enzyme Of Two Maize Cultivars With Age Of Plant Under Salt Stress

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The proposed research work was conducted to determine the effect of age on total soluble protein content and catalase content from seed germination and leaves of two cultivars of Maize (MP-6525 & MP-6142) under salt stress. The seeds were sown in pots and stress of different concentration (50 mM, 75 mM, 100 mM, 125 mM and 150 mM) was created. Salt of sodium (NaCl) was used to induce salt stress. Growth of the two cultivars was assessed by biochemical marker i.e. total soluble protein content (TSPC) and catalase enzyme content. There are numerous antioxidant enzymes but the present study was focused on catalase. The amount of enzymes was estimated on the basis of activity from freshly collected leaf samples weekly. After the analysis of enzymatic activity, the plant was uprooted after seven weeks. MP-6525 overall showed better results in all stress conditions as compared to MP-6142. For total soluble protein content, in seed germination and leaves MP-6525 at 125 mM NaCl stress showed moderate positive correlation. For catalase content it was found that MP-6525 at 75mM NaCl stress with the age of plant has highest R^2 value among all the values. While at germination stage catalase showed sensitivity to salt stress. But MP-6525 showed constant increased in TCC from 125mM to 150mM. It can be concluded that both cultivars contain different type of soluble protein content and catalase content. But MP-6525 found to be the best in quality having its higher total soluble protein content and catalase content at each stage as compared to the MP-6142.

4.26-P Antioxidant and phytochemical studies of *Ipomea batatas* (Sweet potato) leaves extracts

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Plants play a vital role for survival of *Homo sapiens* because the nature is still the ideal hotspot for wellbeing advancement and for supplementation of safe medications. Natural products have proved of great impact on human health traditionally and significantly. In the present study of *Ipomea batatas* belonging to family Convolvulaceae, leaveswere evaluated for antioxidant and phytochemical studies. The qualitative analysis of phytochemicals e.g., alkaloids, flavonoids, saponins, steroids, tannins and terpenoids were carried out. The leaves were extracted by marceration with n-hexane, chloroform, ethyl acetate, n-butanol and 80% methanol. The total flavonoids and total phenolic content were assessed by spectroscopic techniques. Antioxidant potential was measured by DPPH free radical scavenging and linoleic acid oxidation assays. Results were analyzed statistically. From the present study it was concluded that leaves of *Ipomea batatas* could be used as a source of natural antioxidants and phytochemicals.

4.27-P Phytochemical and antioxidant studies of *Prunus domestica* (Plum) leaves extracts

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Plants being producers exhibit a wide range of pharmacological activities and have the potential to be developed into drugs. In the present research work, the leaves of *Prunus domestica*, a member of family Rosacea, renowned for its great medicinal importance, were analyzed for their phytochemicals and antioxidant activity. The plant leaves were extracted by maceration with increasing polarity based solvents i.e. n-hexane, chloroform, ethyl acetate, n-butanol and methanol. The qualitative analysis was carried out for phytochemicals like alkaloids, flavonoids, saponins, steroids, terpenoids, cardiac glycosides, phlobatannins, saponins and tannins. In order to measure the antioxidant potential, the total flavonoid and total phenolic contents were analyzed by spectroscopic techniques. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activities along with percentage inhibition of peroxidation in linoleic acid system were also carried out. BHT was used as a positive control in percentage scavenging assay. The reducing power of extracts was also evaluated and results were analyzed statistically.

4.28-P Serum Mineral And Cell Mediated Immunological Profiling In Male Layer Breeders Following Dietary Regime Alteration

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In birds, for periodic replacement and shedding of feathers, molting is a natural phenomenon. Induced molting in poultry industry being economical and important managemental tool has been used extensively to rejuvenate internal organs of birds to improve production efficiency for next production cycles. This study was aimed to investigate the serum mineral and cell mediated immunological profile in male layer breeders following dietary regime alteration. For this study fifty nine weeks old retired breeder males (n=270) were purchased and maintained. One week post acclimatization birds were forced molted by ZnO (3g/Kg diet). After completion of molting, birds were randomly divided into six groups (45birds/group). First group served as control, birds in other groups were fed diet supplemented with vitamin C (500 mg/Kg), vitamin E (90 mg/Kg), probiotics (Protexin®; @ 0.05 g/Kg of feed), protein (12%) and a combination of all above supplementations, respectively. After semen production, serum sampling was done for five consecutive weeks at one week interval to analyze immunological parameters and serum minerals profile. Result showed that macrophage engulfment percentage, delayed type of hypersensitivity response and concentration of serum minerals were significantly increased in vitamin E supplemented group, following vitamin C supplemented groups. In conclusion vitamins (E and C) and probiotics are helpful in improving cell mediated immunological profile, while vitamin E and C, were found helpful in strengthening of serum mineral profile in male layer breeders, for efficient production. However, in this regard vitamin E is found to be the best option.

4.29-P Evaluation of Hematopoietic Effect of *Angelica sinensis* Polysaccharides in Albino Rats Treated with Lisinopril

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Anemia is a common side effect of prolonged use of lisinopril, an antihypertensive agent, due to decreased production of erythropoietin[1]. *Angelica sinensis* polysaccharides (ASP) possess hematopoietic properties by stimulating erythropoietin production in vivo[2]. The present project was designed to evaluate hematopoietic effects of ASP in alleviating lisinopril-induced-anemia in albino rats during 28 days treatment period. Whole blood and tissue samples were collected at 28th day for hematological and histopathological studies of liver and kidney. We found that lisinopril significantly decreased hematocrit value, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) indicating microcytic hypochromic anemia which was restored when animals were co-treated with recombinant human erythropoietin (rhEPO). ASP alone treatment did not affect the Hb contents, RBC count, MCH level suggested that ASP did not possess hematopoietic effect in contrast to previous literature. Lisinopril+ASP treatment dramatically decreased the RBCs count, Hb contents, Hct level and MCV levels significantly than lisinopril alone treatment suggested some adverse pharmacokinetic or pharmacodynamic interactions in combined therapy. Histopathological examination revealed that lisinopril alone or with rhEPO exerted toxic hepatic and renal effects while ASP along with lisinopril ameliorated these effects. Unexpectedly, lisinopril+ASP treatment exacerbated decreased RBCs count, hemoglobin, hematocrit and MCV levels indicating adverse pharmacological interactions at bone marrow level. Studies are underway to evaluate the expression of LIM-homeobox domain transcription factor Lhx2, recently shown to fine-tune the balance between self-renewal and differentiation of hematopoietic stem cells[3], following combined treatment with lisninopril and ASP.

4.30-P Synthesis, Quality Control And Biological Evaluation Of ^{99m}tc-Lincomycin In Infection Induced Animals

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A complex of lincomycin was synthesized with technetium-99m. The synthesis was carried out by using $SnCl_2.2H_2O$ as reducing agent and ascorbic acid as stabilizer. The effect of various parameters such as amount of ligand/reducing agent, pH value and reaction time on radio labeling process was studied. The characterization of the ^{99m}Tc-Lincomycin was performed by HPLC (Fig. 1) and electrophoresis Biodistribution studies were carried out by analyzing the model of bacterial infectious rats (Sprague-Dawley). The uptake of infectious lesions at different time interval was also studied by using scintigraphic technique. The complex showed effective target to non-target ratio for various inflammatory or infectious lesions (Fig. 2). The ^{99m}Tc-Lincomycin effective binding to living bacteria and could be used successfully as an infection imaging agent.

4.31-P Evaluation of phytochemical, antioxidant and cytotoxicity studies of *Carica papaya* (Papaya) seeds extract and its use for stabilization of vegetable oil

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*Carica papaya*has a great medicinal importance. *C. papaya* is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber. In view of its tremendous medicinal properties, seeds of*Carica papaya*plant have been selected in the present research work for evaluation of phytochemical, antioxidant and cytotoxicity studies. The seeds were extracted by maceration with increasing polarity based solvents i.e. n-hexane, chloroform, ethyl acetate, n-butanol and 80% methanol. The qualitative analysis of *Carica papaya* seeds was carried out for phytochemicals like alkaloids, flavonoids, steroids, terpenoids, cardiac glycoside, phlobatannins, saponins and tannin. Results of alkaloids, tannin, saponins and terpenoids carried out positive but the results steroids, phlobatannins, andcardiac glycoside were negative. TPC and TFC assessment was also carried out. Antioxidant potential was measured by DPPH free radical scavenging and linoleic acid oxidation assays. The extracts will also be used for the stabilization of vegetable oils.

4.32-P Anti-oxidant, anti-diabetic, anti-microbial and enzyme inhibitory activities of *Ricinus communis*

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Medicinal plants are very important in whole world. Due to their effective results herbal medicines are used in whole world. *Ricinus communis* is one of the medicinal plants. Its different parts like stem, root, leaves and seed have gained great importance because of high medicinal properties. *Ricinus communis* (Castor) is a very useful medicinal plant and it is traditionally used as antidiabetic, anti-inflammatory, antiasthmatic, anticancer, antimicrobial, antiimplantation, antifungal, central analgesic, wound healing, laxatives, and cathartic. *Ricinus communis* is a weed whose leaves and seeds are widely studied and used in different medicines. Potentials of roots of *Ricinus communis* has been undertaken in present work by determining its activities like enzyme inhibitory, anti-microbial, anti-diabetic and anti-oxidant. For determining different activities seven extracts were prepared from roots of *Ricinus communis*. These extracts were methanol, ethanol, aqueous, butanol, n-hexan, chloroform and ethyl acetate. For determining the antimicrobial activity the nutrient agar was used to prepare the media. The *Bacillus subtilis* and *Staphylococcus aureus* were used as strain to check the antimicrobial activity. The rifampicin was used as synthetic inhibitor. The methanol and ethanol extracts were bacteriostatic but remaining all extracts gave good zones of inhibition. Their zones of inhibition were not more than synthetic drug but they were quite near to it. In case of *Bacillus subtilis* the zone of inhibition was 33mm and for *Staphylococcus aureus* 43mm shown by synthetic drug. The aqueous extract gave 29mm zone of inhibition for *Bacillus subtilis* and n-hexane extract gave 33mm zone of inhibition for *Staphylococcus aureus* which were quite near to synthetic inhibitor. The zones of inhibition of other extracts were also good but they were less than these two extracts. The oxidative stress is the cause of many diseases. The antioxidant activity was determined by different methodologies. For determining total phenolic contents Folin-Ciocalteu reagent technique was used. Aluminum Chloride colorimetric method was used for determining the total flavonoid content. For determining the antioxidant activity, DPPH radical scavenging activity was used. The seven extracts gave good antioxidant activity. The glycation occurred when glucose or fructose form bond with lipid and protein to form advanced glycation end products (AGEs). Which cause the fibers to become stiff and malformed. The antiglycation potential activity was processed with the help of authorized methodology. Bovine serum albumin, glucose, synthetic inhibitors niacin, metformin and para amino benzoic acid were used to check the activity. All the extracts gave good results in it too. For determining inhibition of enzymes the enzyme inhibitory activity was done. The bacterial alpha amylase was used to analyze this. The activity was checked after 30minute, 60minute, 90minute and 120 minute. It was seen that all extracts gave enzyme inhibitory activity.

4.33-P Management of Anthracnose Disease by Extracts of *Euphorbia hirta L.*

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The present study was intended to evaluate antifungal compounds potential of *Euphorbia hirta* L. against *Colletotrichum gloeosporioides* Penz., the casual agent of anthracnose disease of mango. Methanolic extract of *E. hirta* was tested *in vitro* against the test fungus. Different applied concentrations (1.5-3%) showed pronounced affects in retarding colony diameter of *C. gloeosporioides*. Maximum reduction (28%) was showed by 2.5% concentration; however 3% concentration was also effective in reducing the test fungus growth upto 22%. Phytochemical analysis of the methanolic plant extract of *E. hirta* indicated the presence of glycosides, flavonoids, alkaloids, phlobatannins, tannins and coumarins. This methanolic extract was subjected to bioassay guided fractionation due to the presence of these secondary metabolites. *In vitro* bioassays with different organic fractions showed that chloroform fraction was found the most effective as its two concentrations causing 73% and 77% growth inhibition. This effective fraction was then selected for GC-MS analysis. Fourteen phytochemical compounds were identified from the chloroform fraction of *E. hirta*. The major constituents namely linoleic acid, oleic acid and stearic acid along with some minor constituents were detected. These compounds might be responsible of antifungal activity of *E. hirta.*

4.34-P Evaluation of phytochemical, antioxidant and cytotoxicity studies of *Ficus carica* leaves extracts

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The chemical substances in plants that may perform important functions in the body have been designed as the phytochemicals. Phytochemicals have an enormous importance in medicinal sciences as they have a natural origin. So, there is an intense need for the evaluation of plants for their imperative properties. In this study, *Ficus carica,* a member of mulberry family, were analyzed for phytochemical, antioxidant and haemolytic studies. The leaves were extracted by marceration with chloroform, n-hexane, methanol and ethyl acetate. The qualitative analysis were carried out for phytochemicals like alkaloids, flavonoids, steroids, saponins and tannis. The total flavonoids and total phenolic content were analyzed by spectroscopic techniques. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity were be carried out. The cytotoxicity study was carried out by haemolytic activity against human erythrocytes. The results were statistically analyzed

4.35-P Extraction of humic acid from indigenous low-rank coal and its evaluation as raw material for Zn organomineral biofertilizer

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Pakistan is rich with low grade indigenous coal resources. To date, the main emphasis of coal resources is laid down on the energy production while the non-energy utilization (soil conditioner) is unexplored. Therefore, this study attempted to extract, humic acids from Abbotabad coal to use it as biofertilizer. Humic acid was extracted from the coal samples by potassium hydroxide assisted by organic solvent of n-butanol using ultrasonic bath. The maxium yield (64.76±0.36) % of humic acid was obtained at optimized conditions (KOH (0.75 M), 1M of n-butanol, 8 hours standing time and given ultrasonic radiation for 20 minutes).The extracted humic acid was classified as type "b" of humification index value (9.27) and coefficient of humic acids (0.84) observed by UV-Vis spectroscopy. The elemental analysis and FTIR spectroscopy, suggest the humic acids of the Abbotabad lignite mixture of aromatic/aliphatic characteristics, a great amount of carboxylic groups and a smaller number of N- containing groups. Organo mineral complexation of these humic acid was carried out with 0.1M $Zn(NO_3)_2$. Further, decomplexation titration curves of humic matter saturated with Zinc ions demonstrated that novel organo- zinc fertilizing materials may develop which can retain zinc in a soluble form within a wide pH range.

4.36-P Amelioration of Acid Stress in *Zea mays* L. via Selected Exogenous Applications

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Physiological mechanisms mediated by potassium and phosphate contribute for better plant growth and metabolism. The principal goal of this paper was to assess amelioration potential of KH_2PO_4 against acid stress in plants. To accomplish this, it was planned to assess the changes in activities of chief antioxidant enzymes, photosynthetic pigments and level of some metabolites in relation to acid stress in maize seedlings. Seedlings were exposed to three pH levels (2, 3 and 7) with and without KH_2PO_4 (KP). High levels of stress significantly depressed all growth characteristics, biomass, chlorophyll a and b, carotenoids, leaf area, activity of superoxide dismutase (SOD; EC 1.15.1.1), root phenolics and root flavonoid. Stress did not significantly affect ratio of chlorophyll a to b. Moreover, at all pH levels stress significantly enhanced the activity of peroxidase (POD; EC 1.11.1.7), levels of H_2O_2 and shoot flavonoids. KP treatment improved photosynthesis of maize plants under acidic conditions, that was further associated with an increase in chlorophyll a, root phenolics, flavonoids and activity of SOD. Content of H_2O_2 was lowered by treatment in stressed plants indicating comparatively efficient scavenging of reactive oxygen species (ROS) following a significant enhancement of antioxidant enzyme (SOD) activities. It suggests that KP treatment could improve vigor of plants grown under low pH stress.

4.37-P Therapeutic value of Phytochemical in the treatment of different neoplasm

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Cancer is specified as a broad group of various infections usually characterized by persistently uncontrolled and abnormal cell production, where cell proliferation occurs via mitotic deregulations and thus leads to overgrowth of cancerous cells. These cells in malignance may migrate to other body parts in form of tumors and metastasize in blood stream and lymphatic system. The developing countries contribute approximately 53% of disease incidences with 56% mortalities due to these illnesses, while percentage proportion of new cases is expected to rise by 73% as compared to developed ones (29%) in 2020. Some of these treatment regimens include chemotherapies, radiotherapies, immunotherapies, surgeries etc. These therapies, individually as well as in combination, have proved to be significantly effective (e.g. chemotherapy plus surgery) against various cancer lines. However, survival percentage greatly varies depending upon form, stage and site of the disease in addition to age of patient. Moreover, many difficulties and deficiencies including probable toxicity and damage to other non-targeted tissues and multidrug resistance formation during cancer therapy also limit and/or compromise the effectiveness of some of these therapeutic processes (like chemotherapy traditionally all over the world prior to the advent of allopathic medicines. The natural plant have great role in medicinal products not only possess broad spectrum effects but also ease patients from possible side effects as anti-HIV and anticancerous agent as well as for treating fever, neuralgia, epilepsy, malaria, sprains, cough, dyspepsia. The thousands of plants derivatives including camptothecin, flavopiridol, paclitaxel, combretastatin, betulinic acid, vincristine, vinblastine teniposide, etoposide, irinotecan, topotecan, vincristine, vinblastin and taxol (plant derived drugs) have been screened and modified synthetically to eliminate the complications and increase their anticancer selectivity and efficacy by inducing apoptosis via antioxidant production, depolarization and trans-membrane potential in mitochondria modulating immune system, down-regulating the tumor triggering anti-apoptotic and up-regulating the pro-apoptotic genes and proteins especially BCL-2 family.The wogonin, flavokawain B, Sclerocarya birrea, Limoniastrum guyonianum, Pleiocarpa pycnantha , Excoecaria Agallocha, olive leaf, Ferulago angulata Boiss and Strigolactone analogues were recorded highly anti-neoplastic activity against Leukemia, Breast, liver, cervical, colon, lung, skin, Gastric and prostate cancer cell lines at different IC_{50} value.

4.38-P Phytochemical Evaluation of *Momordica charantia* and *Syzygium cumini*

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Various herbal medicines and natural drugs have been used against different pathological disorders for their lower side effects. There are different medicinal properties have been observed in various parts of the plants. These medicinal properties include treatment of diverse group of diseases. *Momordica charantia* and *Syzygium cumini* are edible plants and commonly used in traditional medicine since ancient times. *Momordica charantia* and *Syzygium cumini* are used in traditional medicine for various ailments. Present study was conducted with the aim to assess phytoconstituents and antioxidant activities of ripe fruits of *Momordica charantia* and *Syzygium cumini.* Total phenolic and flavonoid contents were 14.03 mg GAE/g and 6.22 CE mg/g in aqueous extracts of *Syzygium cumini*. For *Momordica charantia*, 23.6 mg GAE/g and 10.01 CE mg/g phenolics and flavonoids were detected. About 50% DPPH radical scavenging activity was assessed for *Momordica charantia* and 73% was exhibited by water extracts of *Syzygium cumini.* Both the tested plants showed reasonable chemical compositions that may play a vital role in a drug discovery program for the generation of some significant drug compounds.

4.39-P Antifungal potential of secondary metabolites of *Pseudomonas syringae* against *Macrophomina phaseolina*

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During recent years, biological management of plant disease through exploring role of plant growth promoting rhizobacteria is encouraging due to hazardous constraints imposed by chemical methods. Presently, *in vitro* antifungal potential of secondary metabolites of *Pseudomonas syringae* was assessed against well-notorious soil borne phytofungus *Macropomina phaseolina*. In broth, ten various concentrations ranging from 2, 4, 6, ---------- 20% of secondary metabolites of *P. syringae* were tested their efficacy against pathogenic fungus using 2% malt extract growth medium. Percentage growth inhibition and biochemical parameters like total protein content (TPC), activity of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) of pathogenic fungus were assessed at different intervals i.e., 48, 72 and 96 hours. Results showed that secondary metabolites of *P. syringae* at concentrations range of 2-10% significantly reduced the fungal growth by 70-99%, while the fungus was unable to grow beyond this concentration range. Furthermore, TPC, CAT, POX and PPO were significantly increased with increase in concentration of secondary metabolites at different intervals. It is concluded that *P. syringae* can be used as potential bio-control agent against *M. phaseolina*. Further study may be conducted to check the antifungal potential of *P. syringae in vivo* to be used as a bio-fertilizer for management of fungal diseases for sustainable crop production.

4.40-P Variation in Biochemical and antioxidant attributes of *Raphanus sativus* L. in response to foliar application of plant leaf extracts as *PGRs*

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The present study was conducted to investigate the variations in biochemical, antioxidant and nutritional attributes of Radish (*Raphanus sativus* L.) under priming treatments of plant leaf aqueous extracts including mulberry leaf extract (*MLE*), brassica leaf extract (*BLE*), sorghum leaf extract (*SLE*) and moringa leaf extract (*MoLE*) as natural growth regulators. Samples of radish were harvested at peak vegetative stage and evaluated for a group of analysis. Our findings showed that total phenolic contents (TPC) were 29.90±1.47 to 48.02±1.38 (GAE g/100gm of dry weight), IC_{50} value for radish 28.14±1.254 to 33.65±1.346µg/ml) and the antioxidant activity was highest which were treated with Moringa 3% (6 hours). Reducing power of samples was 0.040±0.20 to 0.857±0.06 mg/ml. In further, Proline contents of leaves of radish ranged from 0.196±0.016 to 0.364±0.028 and were highest in the leaves which were treated with Moringa 3% (6 hours). Chlorophyll *a* and chlorophyll *b* contents were 0.622±0.024 to 3.718±0.17 mg/100 g FW. Ash contents was found 6.60±0.54 to 28.50±0.76 g/100g FW and crude fiber contents 15±0.432 g/100g FW to29±0.504 g/100g FW. The overall observations varied significantly among the applied PGRs but Moringa 3% (6hours) showed the best performance among the applied PGRs. The data thus obtained was analyzed statistically by using ANOVA techniques.

4.41-P Evaluation of Fungicidal Potential of *Nicotiana tabacum* l. and *Ocimum sanctum* l. Against *Aspergillus flavus* and *Aspergillus niger*

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Plants of the families Lamiaceae and Solanaceae are of great significance from medicinal point of view. They have been used for centuries for many different purposes as plants of both families exhibit marvellous therapeutic potentials. The present study was undertaken to evaluate the antifungal activity of a locally found and ethno-botanically important plants *Ocimum sanctum* Linn. that belongs to family Lamiaceae and *Nicotiana tabacum* Linn. which is a plant of family Solanaceae. The reason for selecting those plants is that both are widely used plants all over the world and rich in secondary plant metabolites i.e phytochemicals that play role in enhancing their pharmacological activity. The first step was the extraction of powdered plant material in methanol of both plants in order to get their crude extracts. Extraction was carried out by means of maceration technique followed by fractionation (liquid-liquid partitioning) as a result of which all polar and non-polar solvents get separated according to their solubility. For fractionation we make use of solvents of analytical grade such as n-hexane, dichloromethane, ethyl acetate, butanol and methanol. Qualitative phytochemical tests were carried out by the use of various chemicals like aluminium chloride ($AlCl_3$), potassium hydroxide (KOH), ferric chloride ($FeCl_3$), conc. sulphuric acid (H_2SO_4) to confirm the absence or presence of flavanoids, glycosides, phenolics, saponins, steroids, tannins and tri-terpenes, all of them account for the antifungal potential of respective plant species. For carrying out antifungal activity two test fungal strains belonging to genus Aspergillus were selected i.e *A. niger* and *A. flavus* against which the effect of each fraction for both plants was checked. In *O. sanctum*, n-hexane fraction is most effective (100%) at conc. of 0.3% against *A. niger* while minimum inhibitory effect has also been given against *A. niger* at conc. of 0.7% i.e 9% by crude extract. On contrary to *O. sanctum* in which both maximum and minimum inhibition was given by *A. niger*, in *N. tabacum A. flavus* is dominant. Least inhibitory concentration was 3% given by crude fraction at 0.1% against *A. flavus* while on the other hand maximum control for fungal growth has been recorded in butanol fraction at 96% also at the same concentration of 0.1% against *A. flavus*. Hence these plants can be used for manufacturing drugs to preserve our cereals and to treat fungal infections as both possess significant antifungal activity.

4.42-P Ascorbate Peroxidase and Soluble Protein Content in Two Cultivars of *Zea mays* l. Under Chromium Stress

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The proposed research work was conducted to determine the soluble protein content and antioxidant enzyme activity in two cultivars of *Zea mays* L. under chromium metal stress. The seeds were sown in earthen pots and stress was applied. Salt of chromium ($K_2Cr_2O_7$) was used to induce the heavy metal stress and plants were allowed to grow for seven weeks. There are numerous antioxidant enzymes that are part of plant defense system but the present study was focused on ascorbate peroxidase (APX). The amount of enzyme was estimated on the basis of activity from freshly collected leaf samples and seeds and correlation between the amount of stress with total soluble protein content and ascorbate peroxidase of the plants was evaluated. Results of TSPC in seeds and leaves of MP-6525 were with maximum TSPC in seeds upto 19.4 mg/g at 40 ppm as compared to its control (18.72 mg/g), showing a weak correlation (R^2= 0.320) with stress and in leaves it was reduced in last week upto 50 ppm (11.08 mg/g) as compared to its control (11.63 mg/g), giving a weak negative correlation (R^2= 0.221) that shows its tolerance capacity for chromium. Whereas in seeds and leaves of MP-6142, highest TSPC in seeds was at 10 ppm (18.99 mg/g) as compared to its control (18.17 mg/g), exhibiting a weak negative correlation (R^2= 0.006) with stress and in leaves it was reduced in last week upto 50 ppm (6.52 mg/g) as compared to its control (9.67 mg/g), with a stronger negative correlation (R^2= 0.422) with stress. Maximum APX activity in seeds and leaves of MP-6525 was that in seeds the extreme APX content was obtained at 50ppm (0.0474 mg/g) as compared to its control (0.049 mg/g), showing a weak positive correlation (R^2= 0.002) with stress and in leaves it was maximum in last week at 40 ppm (0.0491 mg/g) as compared to its control (0.036 mg/g), giving a strong positive correlation (R^2= 0.719) with stress. In MP-6142, APX activity in seeds was highest in the last week at 40 ppm (0.0471 mg/g) as compared to its control (0.031 mg/g), exhibiting a quite a strong positive correlation (R^2= 0.632) with stress. It has been concluded from the results that MP-6525 showed better tolerance capacity for chromium stress than that of MP-6142 by lesser TSPC and more production of APX enzyme. Hence, MP-6525 can be used for cultivation purposes in Cr-polluted areas.

4.43-P Salt Stress Alleviation By Thiourea In Maize Hybrids: Evidence From Ionic And Nutrient Estimations

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Thiourea is a potential plant growth regulator and proved to be very effective in improving tolerance in a variety of plants. This study was arranged to find out level of salt stress amelioration by thiourea application on maize hybrids at both cellular and whole plant level. Thiourea levels (0 µ*M* and 400µ*M*) were applied on two hybrids (DK6789 and 33M15) through sand application to 15-day old controlled and salt stressed (0m*M* and 120m*M* NaCl) plants, and same hybrids were used for callusing attributes with similar thiourea levels under normal and saline medium conditions. Salt stress severely affected growth attributes at whole plant and callus level. Salt stress caused damaging effects as substantial suppression of essential nutrient uptake as decreased K^+, S, NO_3^{-2}, P and Ca^+ by increased accumulation of Na^+, Cl^- and anthocynin in shoot, root and callus of both maize hybrids. Thiourea significantly improved *in vivo* and *in vitro* growth and morphological attributes of shoot, root and improved callus quantity, quality and regeneration by improving essential ion uptake as increased K^+, Ca^+ and NO_3^{-2} and decreased Na^+ and Cl^- accumulation under control and saline. From the changes in the growth and physiological attributes of maize, it is concluded that thiourea application owes a great potential to alleviate the deteriorating effects of salt stress on maize. So, thiourea can be used for improving growth in moderately saline soils. This could be a great achievement towards better maize genetic transformations for development of resistant crops.

4.44-P Therapeutic Significance Of Dairy Derived Bioactive Peptides

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Many food proteins can exert a physiological action, either directly or, after their degradation, in the form of fragments. Peptides represent a quite heterogeneous class of compounds and their characteristics deeply depend on the amino acidic composition and on the length of the chain. Bioactive peptides are described as food-derived components (genuine or generated) that, in addition to their nutritional value, exert a physiological effect in the body. They may be released by proteolysis during gastrointestinal transit or during food processing. Recent studies have shown that milk proteins can yield bioactive peptides with opioid, mineral binding, cytomodulatory, antihypertensive, immunostimulating, antimicrobial and antioxidative activity in the human body. Bioactive peptides are encrypted in milk proteins and are only released by enzymatic hydrolysis in vivo during gastrointestinal digestion, food processing or by microbial enzymes in fermented products. At present significant research is being undertaken on the health effects of bioactive peptides. A variety of naturally formed bioactive peptides have been found in fermented dairy products, such as yoghurt, sour milk and cheese. In particular, antihypertensive peptides have been identified in fermented milks, whey and ripened cheese. Some of these peptides have been commercialized in the form of fermented milks. Bioactive peptides have the potential to be used in the formulation of health-enhancing nutraceuticals, and as potent drugs with well defined pharmacological effects.

4.45-P Impact of Copper on growth and biochemistry of charcoal rot pathogen-*Macrophomina phaseolina*

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Macrophomina phaseolina is devastating soil-borne plant pathogenic fungus responsible for causing disease over 500 plant species. Generally, copper-based fungicides are used to manage the pathogen, such a management not only deteriorates the plant health but effects the environment as well. *In vitro* tolerance of *M. phaseolina* in terms of growth, physiology and metal uptake capacity against different doses of copper [Cu(II)] metal was assessed. Initially, screening trials were conducted to determine the tolerance level of the fungus for three salts of Cu viz., nitrate, chloride and sulphate. To accomplish this task, seven different concentrations viz. 25, 50, 75, 80, 85, 90 and 100 ppm each of three salts of metal were added in malt extract agar medium along with fungus inoculum. Amongst three salts, the maximum inhibition in radial growth of the fungus was observed with increasing concentrations of copper salts in order of: $CuSO_4$> $CuNO_3$> $CuCl_2$. Later experiments were performed in malt extract broth with four different concentrations (25, 50, 75 and 100 ppm) of copper sulphate to evaluate growth inhibition, metal accumulation, protein and antioxidant enzymes i.e., catalase, peroxidase, nitrate reductase, superoxide dismutase and polyphenol oxidase activity at three time intervals (48, 72 and 96 hours) in the fungus biomass. Results showed that when metal concentrations were increased the fungal biomass was drastically declined by 60-100% with net metal accumulation of 50%. Increase of up to 50 to 200% in total protein contents and antioxidant enzyme activities indicated activation of fungal defense response against increasing Cu concentration from 25-100 ppm with incubation period from 48, 72 and 96 hours over control. It is concluded that the high concentrations of Cu are required to control growth of *M. phaseolina*, which could be very noxious not only for the host plant but also for the other biota.

4.46-P Preparation and Experimental Evaluation of Vero Cell Adapted Food Based Thermo-stable Newcastle Disease I-2 Vaccine for Wild Birds

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Newcastle disease (ND) is a nightmare of avian birds. There are a number of different bacterial and viral diseases which have devastating effect; the most eminent is Newcastle disease. Vaccination is the only way to control viral infections. Commercially, there are many heat labile NDV vaccines available in the market which requires cold chain to maintain their efficacy from production to inoculation. Keeping in view, current project was designed to develop a Vero cell adapted food based thermo-stable ND vaccine to protect wild birds. For this purpose, Vero cells were grown in $25cm^2$cell culture flasks. 0.25 ml I-2 NDV strain was inoculated into healthy confluent monolayer of Vero cells. After, 20^{th} passage of virus, cytopathic effects like syncytial formation, aggregation of cells, and detachment of cells were observed. This passage number virus was observed as adapted virus on Vero cells. When the virus was adapted then vaccine prepared by using different food vehicles e.g. wheat, millet and sorghum. The results of biological titration of vaccine showed $3x10^6$ by tissue culture infective dose ($TCID_{50}$). After experimentation, food based thermo-stable Newcastle disease vaccine produced protective antibody titers against challenge virulent NDV infection.

4.47-P Utilization Of Glyphosate As A Sole Source Of Carbon And Phosphorus By Soil Microbes

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Glyphosate is a broad-spectrum and post-emergent herbicide used to eliminate grasses and herbaceous plants. Due to its non-selective nature, it is toxic to non-target organisms consequently there is a need to decontaminate it from contaminated soil. Bioremediation is proved to be efficacious for this purpose pivoting on bacterial isolates with ability to degrade glyphosate efficiently. In this context, capabilities of five bacterial isolates to utilize glyphosate as carbon and phosphorus source under different environmental conditions were evaluated. Among them, a strain showing a significant glyphosate degrading activity was identified by biochemical features and 16S rRNA sequence analysis as *Comamonas* sp. P2. Further experiments were carried out to optimize the growth of *Comamonas* sp. P2 and the glyphosate degradation activity was measured by high performance liquid chromatography (HPLC). HPLC analysis illustrated that *Comamonas* sp. P2 was capable of complete degradation of 500mg/L glyphosate within 3 days. This is the first report on *Comamonas* sp. with a capacity to utilize glyphosate and it can degrade glyphosate concentrations up to 1000mg/L. Furthermore, glyphosate oxidoreductase (GOX) gene from *Comamonas* sp. P2 was successfully amplified which showed 99% homology with already reported GOX genes. Identification of GOX gene indicated that glyphosate oxidoreductase activity was involved in glyphosate degradation. This study demonstrated high potential of *Comamonas* sp. P2 for efficient degradation of glyphosate which can be exploited for reducing soil pollution.

4.48-P Physiological and biochemical analysis of *Alstonia scholaris* leaf galls

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Alstonia scholaris is a small evergreen tree belong to family *Apocyanaceae* and is of great medicinal value. Its bitter milky juice and bark are a source of cure for ulcers, malaria, heart diseases, asthma, leucoderma, tumours and very good for treatment of chronic ulcers, chronic diarrhoea. Unfortunately in the vicinity of University of Punjab, Lahore, the vegetation of this tree is severely affected by an insect, which resulted in leaves deformation with gall like structures. In the present study physiological and biochemical assays of disease and healthy plant were done. The assays included were osmotic potential, membrane stability index (MSI), relative water content (RWC), sugar contents, protein content, peroxidase (PO) activity, catalase (CAT) activity, phenyl analine ammonia lyase (PAL), polyphenol oxidase (PPO), nitrate reductase, phenolic contents, and proline contents. Results showed that drastic reduction in chlorophyll content, PO, PAL, MSI and CAT of diseased leaf as compared to healthy one. Whereas, protein, PPO, sugar, phenol, proline and relative water content was significantly increased in affected leaf over control.

4.49-P Drug Resistant Strains Of *Mycobacterium tuberculosis* Identified Through PCR-RFLP From Patients Of Central Punjab, Pakistan

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The study was carried out to determine, by PCR-RFLP, the magnitude of drug resistance in *Mycobacterium tuberculosis*. The study was carried out on 221 random sputum samples collected from patients and 120 suspected cases of drug resistance. Genetic variation in drug resistant strains was evaluated through PCR-RFLP for isoniazid, ethambutol, streptomycin and ofloxacin. Out of 341 patients, 91.5% were confirmed as *M. tuberculosis* complex infected on the basis of PCR. The random samples revealed resistance in 8.2% cases, while 73.3% of those with suspected drug resistance were found resistant. Among drug resistant isolates, 56.1% were resistant to a single drug, 33.3% to two drugs and 10.6% to more than two drugs. Ofloxacin resistance was observed along with isoniazid, ethambutol and streptomycin in 6.5% cases. Resistance to isoniazid was observed in 61% cases, to ethambutol in 50.4% and to streptomycin in 43.1% cases. PCR-RFLP is a useful molecular technique for the rapid detection of mutations in drug resistant TB patients and may be used to diagnose drug resistance at the earliest.

4.50-P Evaluation of Turnip as Potentail Source of Phenolic Antioxidant

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Plant growth regulators are organic compound, either natural, or synthetic, that modify or control one or more specific physiological processes within a plant, in addition to accelerate or retard the rate of growth or maturation. There for, the present research was designed to invitation the effect of priming application of different plant extracts as a source of natural growth regulator on biochemical and antioxidant attributes of turnip. Experiment was conducted in a sandy loam soil. Priming of *Moringa* leaf extract, *Mulberry* leaf extract, *Sorghum* leaf extract and *Brassica* leaf at two different concentrations (3% and 5%) with 6 hour and 9 hour difference were applied on *Brassica rapa* seeds. The study used a comparison between control (without priming application) and primed seed plant. The leaf sample were collected at peak vegetative stage and subjected to different group of analyses. On consideration of antioxidant activity, the observed total phenol contents (TPC) ranged from 20.53±0.17 to 43.94±0.17 mg GAE /g of FW). DPPH free radical scavenging capacity (IC_{50}) value for turnip ranged from 0.321) value for turnip ranged from 0.321±0.03 to 0.618±0.03ug/mL. Reducing power in the range of 0.091±0.002 to 1.153±0.002mg/mL. Proline contents were found in range of 1.082±0.02 to 4.574±0.02 mg/mL. A study on biochemical parameters have revealed that total chlorophyll contents were in range of 0.876±0.04-2.618±0.17mg/mL and β carotene ranged from 0.422±0.03-0.865±0.02 mg/mL. The physiological parameters such as number of leaves, leaf area, number of primary branching and number of secondary branching were found in the range of 5±0.02 to 7±0.03, to 10.09± 0.03 to 21.54±0.02cm^2, 5±0.02 to 7±0.03 and 0.0 to 3±0.02 g per plant respectively. Moisture contents were found in the range of 80.83±1.75 to 89.82±2.91 %. Ash contents were found in the range of 15.54±0.09 to 18.41±0.07g/100g. Crude fiber contents were ranged from 24.12±0.22-27.79±0.13. The data thus obtained was also analyzed by using ANOVA and results were significantly different in all the treatments. From the results of presents study, it was concluded that all the applied PGRs have shown positive response against control sample; however, *Moringa* leaf extract exhibited the best performance among them.

4.51-P Estimation Of Lead And Cadmium Concentration In Selected Parts Of Sheep And Goat Meat Samples Of Different Zones Of Faisalabad

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Environmental Pollution as the result of rapid industrialization followed by rapid increase in population and with random use of natural resources has intensified in recent decades. Widespread contamination of some ecosystems by heavy metals is also one such health distorting consequence. Metals play vital roles in living organisms. Metals including Cu, Fe, Mg, Cu, Co, Mn, and Zn etc. are considered necessary for human body however, the presence their presence in insufficient or access amounts may cause disturbances in chronic metabolism. There in order to keep body functioning at an appropriate level, the desire range of presence of these metals should be in appropriate range. Selected food diets impart essential role in this context. However, non-essential elements such as Pb, Cd, Ni As, and Cr etc. are toxic because of their presence in living body and can cause intense neurological and biochemical changes inside. Therefore, the aim of present research work was to study the contamination triggered by selected heavy metals *i.e.* Pb and Cd in various parts (liver, heart, lungs and kidney) of sheep and goat meats secured from local markets of six different zones of Faisalabad, Pakistan. After digestion with equinormal amount of HNO_3 and H_2SO_4, each of the 24 samples was run on atomic absorption spectrophotometer along with desired standards to measure the ppm amounts of Pb and Cd. A group of analyses have shown that the higher amounts of both the metals were found to be present in kidneys of both meat samples with respect to any other organ *i.e.* 7.29 ppm in sheep and 7.90 in goat samples. The variations of targeted heavy metals were found to be different with respect to area of collection and organ as shown by statistical analysis.

4.52-P Assessment Of Nutrient Composition And Trace Metals In Locally Available Fresh Milk Samples

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Buffalo milk is known to be rich in calcium, iron, phosphorous, course proteins and vitamin A contents and lower in cholesterol contents as compared to cows, sheep and goat milk. In addition, it is also blessed with tocopherol (natural antioxidant). Unlike the modern dairy cow, buffalo can thrive without higher intake of concentrated feed. Grass, straw and clover make up the bulk of buffalo diet. Besides this healthy composition, there have been found cases combating cow milk's allergy. The present study deals with the nutrient composition and trace metal analysis of fresh milk samples (half liter packing) collected from different locations of Faisalabad, Pakistan. The milk samples were boiled to get optimized conditions and then freeze (1-4˚C). The optimized samples were further analyzed for mineral assessment. Results showed that the amount of casein, fat and ash was found in the range of 3.91-5.02%, 7.63-11.20% and 0.55-0.91%, respectively. The values depicted by mineral analyses determined via atomic absorption were ranged as: 0.20-0.50 ppm for zinc and 0.02-0.08 ppm for lead. The results were taken after running desired standards and experiments conducted on statically basis.

4.53-P Natural *PGRs*, a greener way to enhance yield and antioxidant potential of turnip (*Brassica rapa*)

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The manipulation of growth and increasing productivity of vegetables can be obtained in a greener way by using natural plant growth regulators (*nPGRs*). In present work *nPGRs* were applied in term of seed priming on turnip (*Brassica rapa*) seeds. Mulberry leaf extract was used as *nPGR* and results were compared with commercial *PGR* (cytokinins). The percentage yield of plant extract in terms of seed priming ranged from 10.20-28.56 % and for cytokinins treated sample it was 11.34 %. Antioxidant activity of plant extract was evaluated by using different *in-vitro* assays such as their total phenolic contents, DPPH• scavenging assay and antioxidant activity in a linoleic acid system and results ranged from 10.20-28.30 mg GAE/100g, 18.13-43.98 (IC_{50} mg/mL) and 39.90-89.19%, respectively. The results obtained were statistically analyzed by using one way analysis of variance (ANOVA). Results explored that *nPGR* can not only reduce the cost of production but also help to maintain higher quality of vegetables because of less use of chemicals.

4.54-P Optimization Of Ultrasound-Enzyme Assisted Extraction Of Phenolics From Mango Peel And Pulp (*Mangifera indica* L.)

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Department of Chemistry, University of Agriculture, Faisalabad

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Mango (*Mangifera indica* L.) is one of the most delicious tropical fruit having important nutritional contents; sugars, polyphenols, fat, dietary fiber, vitamins and minerals. Ultrasound-enzyme assisted extraction technique was used, keeping in view yield, cost of process and environmental aspects. UEAE helps in achieving these goals by enzyme hydrolysis to enhance the recovery of plant bioactives by cell wall-degradation and ultrasound increases solubility of target material in the extractant. Samples were pretreated with ultrasonic and enzymatic treatment and extraction was carried out using 80% ethanol and water. The characterization of phenolic acids was done using HPLC-UV. Total phenolic contents (TPC) of fruit were evaluated using Folin-Ciocalteu Reagent. The amounts of polyphenols in mango peel and pulp extracts were 30.56 and 19.96 mg gallic acid equivalent/g fresh weight. By employing DPPH scavenging assays, the highest antioxidant activity was exhibited by alcalase-peel extract i.e. 98.67%. In TEAC assay, alcalase-peel extract shown the highest scavenging activity (39.37%). Alcalase - peel extract showed highest % inhibition (78.30%) in linoliec acid system and highest reducing power (1.992 abs. at 700nm). The results were reported as Mean ±SEM. The data obtained in response to different enzymatic pretreatment was analyzed using Response Surface Methodology (RSM).

4.55-P Biotransformation of α-Naphthol, Benzoic acid and Benzothiazines using *Candida albicans*

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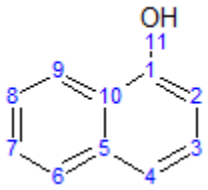
Biotransformation is gaining considerable attention of researchers for attaining chemically impossible products from already available cheaper substances, more excitingly through 'green' process. The cell cultures of different types of microorganisms or their isolates are used in this method to carry out such unique reactions. This work represents reactions of fungal specie, *Candida albicans*. The compounds used as substrate were **1-5**. Various products formed in these reactions will be evaluated for pharmaceutical drugs for the inhibition of different types of enzymes.



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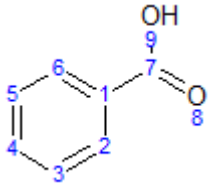

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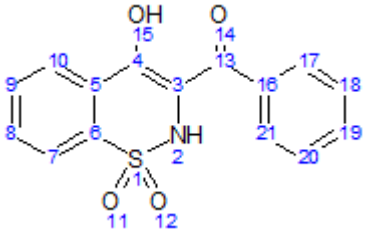

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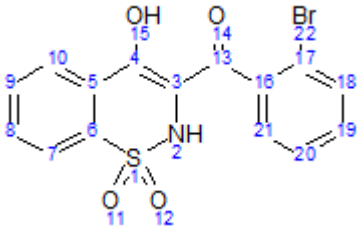

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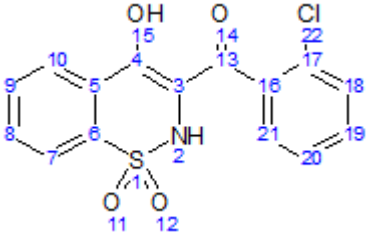

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4.55-P Biotransformation of 3-nitro-*N*-[(3Z)-2-oxo- 2, 3-dihydro-3H-indol-3-ylidene) benzenesulfonohydrazide by Fungi

Ahsan Sharif*, Ejaz Ahmed, Asma Yousaf

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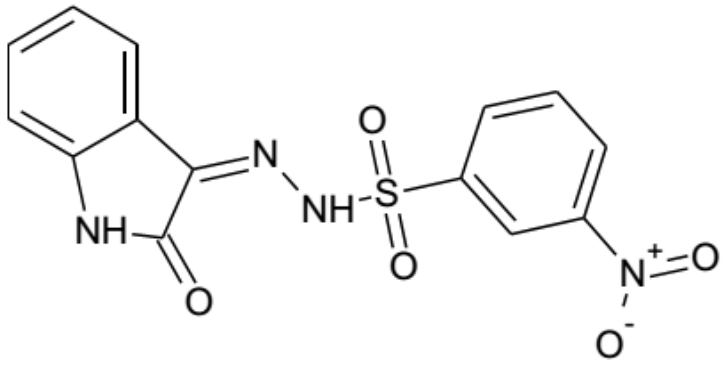
There are number of parameters which influence the bio-degradation of the compound in the media or soil, among these microbial transformations is the important. In the present study we have randomly investigate the role of *Tamrii* spp. On the biotransformation of 3-nitro-*N*-[(3Z)-2-oxo-2, 3-dihydro-3H-indol-3-ylidene) benzenesulfonohydrazide the compound is bio-transformed in number of ways to obtain several compounds. Physical and chemical investigation were performed for the structure elucidation of the compounds and to check the parameters that were responsible for the structural change in the parent molecule. The bio-transformed products were further evaluated for their antimicrobial behaviors.



TRACK 5

BIOANALYTICAL, BIOPHYSICAL, BIOORGANIC AND ENVIRONMENTAL CHEMISTRY

TRACK COMMITTEE

1. **PROF. DR. HAQ NAWAZ BHATTI (FOCAL PERSON)**
2. **DR. RAZIYA NADEEM**
3. **DR. SHAUKAT ALI**

KEYNOTE LECTURES

TRACK 5

K-5.1 Tuning the Size and Surface Chemistry of Metal Nanoparticles/Nanoclusters for Biomedical and Energy Applications

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Nanostructured materials have been recognized as an important class of materials whose properties can be tuned by controlling their nanoscale features. A control over the size, shape and surface chemistry of metal nanoparticles is thus vital to use them for any potential application. We have demonstrated several reproducible protocols to prepare functionalized metal nanoparticles from few atom nanoclusters i.e., subnanometer to over 100 nm in aqueous/organic media with a fair control over their size, shape, and surface chemistry. These metal nanoparticles have been used as building blocks to design/synthesize new nanostructured materials such as composite thin films, porous metal foams, inorganic oxide – metal nanoparticle hierarchically porous composites (heterogeneous catalysts), nanowires, porous microwires, porous nanoballs, nanochains, and nanoscale circuit patterns etc.using template-based (biological and non-biological) and template-less strategies. These functionalized metal/metal oxide nanoparticles/nanoclusters possess interesting optical, recognition and catalytic properties and we arenow focusing on their applications in bio-sensing (especially bacterial detection), bio-imaging, drug delivery, forensic science,solar/fuel cells, environmental remediation, H2 production and catalysis. This talk would thus be an overview of our interdisciplinary research activities to synthesizecustomized nanoscale materials having controlled nanoscale features, unique properties and diverse applications particularly in biomedical sciences and environment.

K-5.2 Spectroscopic and chromatographic evaluation of the wax ester fraction of *Adenanthera pavonina* Oil

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Present study was carried out in order to assess the constituents of *Adenanthera pavonina* wax esters by Fourier Transform Infrared (FT-IR) spectroscopy and Gas chromatography (GC). After extraction of oil from *Adenanthera pavonina* seeds, wax esters were separated from oil by centrifugation and then purified through crystallization process in acetone. The white crystalline solid with melting point of 83-85 ^{o}C was initially characterized as wax esters by FT-IR. Gas chromatography with flame ionization detector (GC-FID) provided the separation of various wax esters from C_{34}-C_{48} including odd and even chain esters. C_{46} wax ester was found to be predominant. For separation, identification and estimation of individual constituents of fatty acid methyl esters and fatty alcohol acetates were carried out by GC-MS after saponification and derivatization of wax esters. GC-MS analysis indicated the presence of saturated methyl esters from C_{l4}-C_{30}. Predominant methyl ester was C_{22}. Wax esters contained the saturated fatty alcohols from C_{14}-C_{28}. The major alcohol was saturated C_{24} fatty alcohol. The results indicated that *Adenanthera pavonina* wax esters have potential to be used in pharmaceutical and cosmetic industry due to its better composition of long chain fatty acids and fatty alcohols.

K-5.3 Gold Metallurgy: Future Prospective

Sadia Ilyas* and Haq Nawaz Bhatti

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Gold recovery is interesting due to its vast industrial applications, high market prices and extensively used precious metal, the sanctuary value attributed to gold during international political and economical crises, and the limited resource of this metal may explain the recent increasing gold share value. The state of art in recovery of gold from primary and spent sources by various metallurgical techniques is highlighted in this study. Current work also provides an overview of past achievements and present scenario of recovery studies carried out on the use of some promising methods which could serve as an economical means for extracting gold. Lucrative and environmentally friendly technologies to recover gold from primary and secondary sources are evaluated.

K-5.4 Recent Developments in Metal-Based Anti-Cancer Drugs; Role of Metal-*N*-Heterocyclic Carbenes Complexes

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Discovery of *cis*-platin as an anticancer drug and its marketing was a landmark in the field of metal-based anticancer drugs. However, its severe side effects confined its applications. Later on, a number of its biologically improved derivatives were synthesized. Only few of them (carboplatin, oxaliplatin, nedaplatin and few more) showed better activity with relatively lower side effects, cleared all the clinical trials and are now being marketed as potential anticancer drugs. A number of transition metals, other than platinum, have been now being studied for the purpose of anticancer drug development. Most of them showed good *in vitro* and *in vivo* results, however, failed at various stages of clinical trials. Recently, coinage metals (silver, gold and copper) have been focussed for the development of metal based anticancer drugs due to their biocompatibility and hence lower side effects. *N*-Heterocyclic Carbenes (NHCs) are a new class of organic ligands which are being employed as suitable metal ion carrier and metal ion delivery ligands at the target sites in a biological system. The current work describes a brief background, introduction, synthesis, characterization and anticancer applications of silver(I)-NHC complexes.

K-5.5 Preparation of Novel Hybrids of Polystyrene Nanoparticles and Silica Nanoparticles-Grafted-Graphite Via Modified Technique for Better Thermal Stability and Efficient Conductivity

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Nanoparticle-based nanostructures have been employed in numerous technological areas, ranging from catalysis and coatings to biomedicine and optoelectronics, depending upon the final properties. We have designed polystyrene nanoparticles through modified nanoprecipitation cosolvent evaporation technique and conventional nanoprecipitation and solvent evaporation. Polystyrene nanoparticles were embedded with silica nanoparticles and graphite to analyze scope of nanoprecipitation cosolvent evaporation. Ultraviolet–visible spectroscopic revealed decreased band gap of polystyrene nanoparticles obtained via nanoprecipitation cosolvent evaporation. Scanning electron microscopic showed uniform morphology of polystyrene nanoparticles and polystyrene nanoparticles-based nanocomposites engendered by nanoprecipitation cosolvent evaporation. X-ray diffraction disclosed presence of crystalline domains due to silica nanoparticles content in amorphous structure. Glass transition temperature was increased from 94 (polystyrene) to 124°C (PSNPs/SiNPs 0.6) and 137°C (PSNPs/SiNPs/G 0.6) with filler loading. Electrical conductivity of PSNPs/SiNPs/G 0.6 was also found to be higher (1.53 S/cm).

ORAL PRESENTATIONS

TRACK 5

5.1 Application of Natural and Charred Orange Peel for the Immobilization of Arsenic (v) from Aqueous Solutions.

Muhammad Abid[1]*, Nabeel Khan Niazi [1,2] Irshad Bibi[1,2] Gulam Murtaza[1], Muhammad Bilal Shakoor[1], Mahwish Mahmood[1], Syed Nabeel Husnain[3]and Yong Sik Ok[4]

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Arsenic (As) is a highly toxic and carcinogenic metalloid. Over 170 million people worldwide and about 100 million people in the Southeast Asia region including Pakistan have been exposed to chronic As toxicity via drinking As contaminated water or consuming contaminated food crops. Therefore, it is imperative to develop the low-cost and environmental friendly filtration technology to remove As from the contaminated water. Biosorption efficiency of natural orange peel (NOP) and charred orange peel (COP) was examined for the immobilization of arsenate (As(V)) in aqueous environments using batch sorption experiments. Sorption experiments were carried out as a function of pH, time, initial As(V) concentration and biosorbent dose, using NOP and COP. Arsenate sorption was found to be maximum at pH 6.5, with 98 % As(V) removal by COP than NOP (68 %). Sorption isotherm data exhibited a higher As(V) sorption (60.9 mg g–1) for COP than NOP (32.7 mg g–1). Langmuir model provided the best fit to describe As(V) sorption. FTIR and SEM combined with EDX spectroscopy analyses revealed that the –OH, –COOH, and –N-H functional groups were involved in As(V) biosorption and the meso- to micro-porous structure of COP sequestered significantly (2-times) higher As(V) than NOP, respectively. As(V) desorption from COP was 10% than NOP 26% up to the third regeneration cycle. The results highlight that this method has a great potential to produce unique ‘charred’ materials from the widely available biowastes, with enhanced As (V) sorption properties.

5.2 Adsorption of Synthetic Dye from Wastewater Using Biopolymers

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Industrial colored wastewater plays an important role in environment pollution. Different techniques are developed for removal of dyes from wastewater. Among them, adsorption techniques are considered to be most efficient, simple and cost effective. In present study, the removal of synthetic dye was carried out in batch mode using biopolymers. These biomaterials can be used as potential alternate to the conventional wastewater treatment materials. The effect of different experimental parameters such as pH, biosorbent dose, initial dye concentration, contact time and temperature were determined to investigate the optimum conditions for attaining the maximum removal. The optimum pH and biosorbent dose was found to be 4 and 0.1 g. It was observed that the maximum dye removal was observed at 45 min. Equilibrium and kinetic parameters were determined by applying different equilibrium and kinetic models. The best fitted equilibrium and kinetic models were found to be Freundlich and pseudo-second-order. Different thermodynamic parameters were calculated to determine the nature and feasibility of adsorption mechanism. It was observed that adsorption of synthetic dye using biopolymers was spontaneous and exothermic in nature. It was found that biopolymer can be used as an efficient, cost effective and eco friendly biomaterial for the removal of synthetic dyes.

5.3 Comparative Antimicrobial and Antibiofilm Activity of Fresh Rose Petals and their Waste

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Rose oil and rose water are being commercially used in manufacturing of many industrial products like perfumes, cosmetics etc. After extracting rose oil and rose water a huge amount of rose waste is being produced which is causing serious environmental pollution. There is great demand to handle this problem. In this project, antibacterial and antibiofilm activity of fresh rose (*Rosa centifolia*) and rose residues was evaluated. Phytochemical screening showed that alkaloids, tannins, resins, saponins, flavonoids, phenolics, carbohydrates and glycosides were present in rose residues. Antibacterial activity was checked by disc diffusion method. Antibacterial activity was evaluated against four bacterial strainsi.e*Escherichia coli*, *Staphylococcus aureus*, *Paturellamultocida* and *Bacillus Subtillus*. Extracts prepared by fresh rose showed higher antibacterial activity against all bacterial strains than rose residue. Both extracts also exhibited antibiofilm activity against *Staphylococcus aureus*. Antibacterial and antibiofilm activities are due to the presence of phytochemicals which retain in residues after rose oil extraction.

5.4 Kinetic and Thermal Characterization of Lipase Isolated from *Penicillium chrysogenum*

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Lipases (EC 3.1.1.3) belong to the class of serine hydrolases that catalyse the breakdown of triacylglycerol to diacylglycerol, monoglycerol, free fatty acids and glycerol in the aqueous medium. This study was aimed to investigate the potential of indigenous fungal strain and agro-industrial residues for lipase production and also to investigate the screened fungal lipase for the enhanced thermal and operational stability. Optimization of different process parameters led to 2 folds enhancement in lipase production. Under optimum conditions an enzyme activity of 5335 U/gds was observed. *Penicillium chrysogenum* lipase was purified to homogeneity by four step purification strategy to achieve 28.88 fold purified enzyme with 13.4 % recovery and 26779 U/mg specific activity. The molecular mass of the homogeneous lipase was 46 kDa as determined by SDS-PAGE. It was optimally active at pH 9.5 and 40 °C. The Michaelis Menten constants K_m and V_{max} of lipase from *Penicillium chrysogenum* for para nitrophenyl palmitate hydrolysis at optimum temperature were 3.33 mM and 232.6 μ mol/mL min^{-1} respectively. The enzyme show a high thermo-stability with half lives of 8.25, 3.2, 1.12 and 0.58 h 40, 50, 60 and 70 °C. The activation energy for denaturation was 81.1 kJ/mol. Exposure to hydrophobic environment (urea solution and organic solvents), did not affect the enzyme significantly.

5.5 Anti-Hyperglycemic Evaluation of N-Cyanomethyl-1,2-Benzothiazine and its Derivatives

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Diabetes mellitus is promptly intensifying worldwide disease and number of patients is predictable to levitate 50% over the next 25 years influencing about 600 million adults. The aim is to investigate the newly synthesized compounds for the two biological activities, to recognize and achieve specific anti-hyperglycemic and anti-bacterial goals that can substantially diminish the morbidity making the effective cure of hyperglycemia and bacterial infections. N-cyanomethyl-1,2-benzothiazine and its derivatives have earned too much consideration in biological research as they possess anti-inflammatory, antimicrobial, anticancer, antitumor and anti-diuretic properties. In present study, six synthesized compounds of benzothiazine were reported to study the influence on diabetic and bacterial diseases. To evaluate the antihyperglycemic potential of N-cyanomethyl-1,2-benzothiazine and its derivatives , alloxan monohydrate; a toxicglucose analogue, was injected that selectively demolish the pancreatic*β*-cells and causes diabetes in experimental albino white rats. The 6 group of rats were assessed at two dose levels of 1 mg/kg and 10 mg/kg of tested compounds as drugs. Both the treated groups exhibited significant hypoglycemia except AD111-Mn when compared to 7^{th} group given standard drug; metformin at regular intervals of 15, 30 and 60 minutes which is evident from Plasma Glucose levels. To further potentiate the role of this compound, antimicrobial activity was also measured using standard disc diffusion assay against four bacterial strains in vitro to establish structure-activity relationship. All compounds especially AD111-Cu demonstrated promising antibacterial activity; though, other compounds revealed moderate to poor antimicrobial activity pertaining to the reference drug Ciprofloxacin against the test strains.

5.6 Removal of Congo red dye from industrial wastewater using composites

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Large quantities of toxic effluents discharged from industries are major sources of environmental pollution. Toxic effluents present in wastewater causing a numerous diseases in living organisms. Therefore it must be treated before use. The present study is to establish an economical, effective and environment friendly process for the removal of congo red dye from wastewater. In this study congo red dye is removed by using composites i-e native (Sugarcane Bagasse), Polyaniline / Sugarcane Bagasse, Polypyrole / Sugarcane Bagasse, Starch / Polyaniline, Chitosan / Aniline Chitosan / Pyrole Composites in batch mode. Different process parameters like pH, biosorbent dose, initial dye concentration, contact time, and temperature were optimized. The pH (4-12), biosorbent dose (0.05g, 0.1g, 0.15g, 0.2, 0.3g), initial dye concentration (25, 50,75,100, 125, 150, 175, 200 mg/L), contact time (05-120 min), and temperature (30˚C-60 ˚C) were optimized for Polyaniline / Sugarcane Bagasse, Polypyrole / Sugarcane Bagasse, Starch / Polyaniline, Chitosan / Aniline Chitosan / Pyrole Composites. The mechanism of data have been analyzed using Langmuir and Freundlich isotherms. The isothermal data followed the Langmuir model. Thermodynamic study were used to estimate different thermodynamic parameters. The results showed the endothermic nature of biosorption process.

5.7 Spectroscopic and Theoretical Studies of Interactions between 4-Acetamidophenol and DNA

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Study of Drug-DNA interaction has become important in the field of chemistry, molecular biology and medicine. The present project was designed to study interactions of 4-Acetamidophenol and DNA extracted from human blood, both theoretically as well as experimentally. Theoretical study of 4-Acetamidophenol was carried out with the help of hyperchem software. Geometry optimization, complete understanding of structure, binding energy, isolated atomic energy, gap between HOMO and LUMO orbitals were obtained from this software. Binding of this drug withDNA was examined by using UV-Vis and FTIR spectroscopic techniques.Hyperchromic andsmall bathochromic shifts were observed due to addition of DNA thus confirming the groovebinding and intercalation mode of interaction between drug and DNA.These observations weresupported by the results of hyperchem as the structure of 4-acetamidophenol consists of planarand non-planar regions. The planer region was observed to be involved in intercalation whilenon-planar region forms groove binding. Binding constants were calculated from the increase inabsorbance due to DNA addition. High binding constant (20.12×10^3 mol^{-1} dm^3) at pH 4.7 ascompared to pH 7.4 (5.32×10^3 mol^{-1} dm^3) indicated that oral intake is preferable over itsadministration via blood veins. Negative value of free energy confirms that the formation of 4-Acetamidophenol-DNA complex is spontaneous at room temperature. From the FTIR spectra itwas observed that at pH 4.7 aromatic C-H and aromatic C=C were involved in intercalationwhile amide C=O was involved in groove binding. Whereas at pH 7.4 aromatic C-H wasinvolved in intercalation and alkaline C-H and amide C=O were involved in groove binding.Experimental and theoretical results were compared and found in full agreement.

5.8 Removal of Malachite Green from Simulated Waste Water by Composites

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Rapid industrialization has resulted in increase in coagulation pollution. Textile are playing a major rolein increasing water pollution, the present study is design to appraises the potential of bio-composites, including Polypyrole, Polyaniline, Starch, Chitosan aniline, Chitosan Pyrole and rice bran biomass for the removal of malachite green dye from simulated water solution. In this study the effect of different process parameters (pH, temperature, biosorbent and biocomposites dose, initial dyes concentration and contact time) was investigated. Effect of surfactants and metal ion concentration on the biosorption process has also been checked out. Different kinetic models (pseudo 1storder, pseudo 2nd order, and intraparticle diffusion modal) has been applied on the contact time data. The equilibrium modals (Langmuir, Freundlich and D-R models etc.) has been applied on the initial dye concentration data. Thermodynamic study has also been carried out. The data, thus was obtained statistically by analysis of variance techniques (ANOVA).

5.9 Investigation of Naproxen Sodium Interaction with DNA by UV-visible Spectroscopy, FTIR Spectroscopy and Hyperchem Modeling

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Investigation of interaction between naproxen sodium (NAPS) and human DNA was done by UV-visible spectroscopy, IR spectroscopy and molecular modeling using Hyperchem 7 software at two physiological pH values (4.7 & 7.4). NAPS showed a considerable interaction with DNA and the binding constants calculated from UV-visible spectroscopy were found very high i.e., $3.50\times10^{+10}dm^{-3}/M^{-1}and1.50\times10^{+6}dm^{-3}/M^{-1}$ at 4.7 and 7.4 respectively. Thermodynamic parameters were determined which showed strong spontaneous binding of NAPS with DNA. Absorption spectroscopic results showed the intercalative or electrostatic mode of interaction. FTIR spectra were helpful in recognition of functional groups involved in interaction as well as in revealing the mode of interaction. Changes in vibrational modes indicated the strong interaction via intercalation. Theoretical calculations and geometry optimization also confirmed the experimental results. And an overall mixed mode of interaction was suggested in this paper however NAPS was found to be strong DNA binding drug.

5.10 Arsenic Biosorption in Aqueous Environment by Using Novel Biowastes

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Arsenic (As) is a naturally occurring metalloid and categorized as a Class A human carcinogen by US-EPA. Geogenic or anthropogenic As-contamination of groundwater is an environmental issue all around the globe. Over 200 million people have been affected through intake of As-contaminated water used for drinking purpose. Several technologies areavailable for the treatment of As-contaminated water but the high cost, energy requirement and sludge production limit their applicability. Nowadays biosorption using solid biowastes (e.g. wheat straw, peels of fruits etc.) called as biosorbents has emerged as one of the promising technique for the removal of As from contaminated water. Low-cost, easy availability and negligible sludge production are the main advantages of biosoroption over other remediation techniques. Hence the current study was conducted to compare biosorption capacity of various natural biosorbents including: corn cob (CC), egg shell (ES), jaman seed (JS), tea waste (TW), water chestnut shell (WCS), water melon rind (WMR) and pomegranate peel (PP) for removal of Asfrom contaminated water. Effect of pH (3–10) on biosorptiveremoval of arsenite(As(III)) and arsenate(As(V)) wasevaluated at an initial As(III) and As(V) concentrations of 4 mg L^{-1} and biosorbent dose of 1 g L^{-1}, keeping equilibration time at 120 min. Arsenic in the filtered water samples was analyzed by using a hydride generation atomic absorption spectrometer (HG-AAS). The optimum pH (from pH study)for sorption isotherm experiments were selected both for As(III) and As(V)and experiments were conducted with initialAs(III) and As(V) concentrations of 0.01–7 mg L^{-1}. Effect of biosorbent dose (1-20 mg L^{-1}) and kinetic studies (1 min to 24 h) were also determined for As(III and V). At the end desorption of sorbedAs(III and V) on biosorbents was carried out in order to reuse the biosorbents by using 0.1 M NaOH. Results showed that WMR and ES displayed maximum As removal of 85% and 78% for As(III) (at pH 7) and As(V) (at pH 5), respectively. Langmuir, Freundlich, Temkin and Dubinin-Redushkevich isotherm models were applied. Langmuir,Freundlich and Dubinin-Redushkevich modelsbest fitted the isotherm equilibrium data. In biosorbent dose effect experiment, 1 g L^{-1}biosorbent dose gave maximum sorption both for WMR and ES.In kinetic study maximum sorption was obtained at 2 h contact time. Arsenic(III and V) was successfully desorbed (upto 98 %) from biosorbents. It is concluded that WCS and ES are viable and cost-effective novel biosorbents for Asremoval from contaminated water.

5.11 GC-MS, LC-MS, Cytotoxicity and Antioxidant Analysis of *Russelia equisetiformis* Extracts and Use of its Extracts for Stabilization of Vegetable Oil

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The use of synthetic antioxidants in food products is mostly discouraged because of their adverse effects. In this work we evaluated the cytotoxicity and antioxidant, of absolute methanol extract and its fractions from the *Russelia equisetiformis* plant. The GC-MS of essential oil and LC-MS analysis of Phenolics in *Russelia equisetiformis* was carried out. The IC_{50} and the % inhibition in linoleic acid oxidation was evaluated. Moreover, it was observed to provide a protective effect in H_2O_2 induced oxidative damage in plasmid pBR322 DNA, indicating that the plant has antioxidant properties. The results of present study revealed that plant may be considered as a good source of natural antioxidants. The antioxidant activity of plant was also studied using sunflower oil as an oxidative substrate. Peroxide value (PV), free fatty acids (FFA), conjugated dienes (CD), conjugated trienes (CT) and *para*-anisidine values were also studied by stabilizing the sunflower oil as oxidation substrate.

5.12 Gas Chromatographic Studies of Soil Humic Acid

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Soil organic matter (SOM) is consist of humic acid (HA) and fulvic acid and is greatly important in maintaining soil fertility, productivity and also in protecting soils from degradation and contamination in particular organic and inorganic chemicals, that may reach the soil, as a result affect their mobility, bioavailability, degradation, phytotoxcity etc. Humic substances are the main organic components of soil, formed through plant and animal tissues broken down by chemical and biological processes. Humic acids are widely distributed organic matter generally found in natural waters, soils, sewage, sediments, peat bogs, shales, lignites, brown coals and other deposits. They are poorly biodegradable, nontoxic and sorbent for toxic metal ions, radio nuclides and organic pollutants. Hence, they are environment friendly organic fertilizers which may be utilized effectively to boost up agriculture production. Conductivity of soil samples ranges between 7.43–30.1 showing the clay-loam type of soil and 7.1 – 8.2 pH values indicate the nature of soil slightly alkaline. The GC analysis indicate that most of the samples contain n-decane with a good concentration except 12, 13, 14 which contain n-heptane. This shows the difference of aliphatic part in structure of soil Humic Acid. The presence of anthracene and 1- methyl phenanthrene in all of the samples and standard except 4, 5, 8, 9, 10, 11 indicating the aromatic nature of these soil Humic Acids whereas the later do not possess aromatic characteristic and differ from others. 2-methylketone was not found in any soil HA sample except standard.

5.13 FT-IR Spectroscopic Characterization of Humic Acids in Agriculture Soils of District Khairpur, Sind Pakistan.

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Humic acids (HA) samples were isolated from agriculture lands of District Khairpur Sindh Pakistan by IHSS method. A comparative study of HA samples and the standard (Aldrich) was carried out by FT-IR spectroscopy. The analytical data reveals the polyfunctional nature of Humic acid especially the presence of oxygen and nitrogen containing functional groups such as phenolic, amides, carboxylic acids, esters and peptide linkages. IR spectra illustrates the characteristic peaks of HAs with slight intensity differences in some regions comparable with the standard. The intensity of peaks found high at 3419 cm^{-1} in all samples except T3, T12 and T18 but T12 showed strong symmetric and asymmetric aliphatic C-H stretching in CH_3 and CH_2 at 2853 cm^{-1}. The weak signals of olefinic/aromatic observed. O-H deformation and C-O phenolic groups 1428 cm^{-1} does not appear in T10, T14 and T15. Although a strong band appearing at 1028 cm^{-1} in all humic acids as well as standard seemed to contain more aliphatic alcohol and carbohydrates related functionalities,

5.14 Application of Natural Dye Extracted from Kashmiri Tea with Various Mordants

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A lab-scale study to promote the eco-friendly approach was conducted while exploring a new source of natural-dyes that was indigenous in Pakistan namely-- kashmiri tea which contains various tannin based coloring compositions. The study was conducted from the standpoint of optimizing the dye-concentration in the extract, selecting the suitable metallic-mordant to obtain the multitude of shades as well as to improve the fastness properties of dyed cellulosic fabric and imparting the anti-microbial properties in the same. Using bleached fabric, various shades with improved fastness properties were obtained in comparatively shorter dyeing time and easily approachable temperature. Copper with pre-mordanting was found as a good mordant to achieve the best results with transition metal ions effect. Deep shades (K/S = 9.20) were obtained under maintained temperature at 85 °C for 35 min of dyeing.

5.15 Designing of Efficient Hole Transport Materials Based on TPA (triphenyl amine) for Photovoltaic Applications

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There is an increased awareness of computational chemistry for the designing of organic/Inorganic compounds which saves cost, labor and time. Its primary objective is to solve chemically related problems through calculation by using different methods and theories. Generally, computational chemistry is employed when a mathematical methods becomes well developed and can be used for implementation on a computer. Density Functional Theory (DFT) is one of the most efficient and frequently used methods. The present research work has been directed to design various organic/Inorganic compounds as an efficient material for photovoltaic applications. In our study we aimed to design organic materials for organic solar cells based on triphenylamine (TPA). Triphenylamine has been used as a building block due to its electron donating ability and cyanide as an electron acceptor while OCH3 group for boosting TPA donating ability. Various theoretical approaches have been employed. For this purpose, ground state geometries with B3-LYP/6-31G along with ZINDO/3-21G levels of theories and their basis sets has been used in Gas Phase. TD-DFT (Time Dependent-Density Functional Theory) has been employed with TD-B3LYP/6-31G along with TD-ZINDO/6-31G for the computation of excited state geometries in Gas Phase. CAM and TD-CAM calculations were also carried out to check the absorption properties of the designed molecules. We have come up with design of materials with excellent photophysical and electronic properties. These materials will be synthesized on later stages and used in the fabrication of efficient solar cells.

5.16 Simulation of UV-Vis Spectra of Phenanthrene Based Medicinally Active Compounds

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Computational chemistry has come to the era of chemistry with multi-dimensional benefits. Without using any analytical technique, the three dimensional structure of chemical specie can be determined. With the use of computational chemistry, different parameters have been simulated include optimization of energy, optimization of structural parameters, calculation of energies of HOMO and LUMO, ionization potential, charge analyses, electrostatic potential mapping, UV/Vis-Near IR and vibrational spectra of individual molecules as well as complexes along with energy on interaction, these are all calculated by low energy paths. Phenenthrene it is the backbone of morphine, which in turn is the backbone of a large number of psychoactive chemicals including antitussives, analgesics, and dissociative drugs and show absorption in the light in the UV/Vis region. UV radiation is alarming environmental risk factor for the development of non-melanoma skin cancer, speeds up the aging and burning of skin. UV is also responsible for the formation of bone-strengthening vitamin D in human. Many pigments and dyes absorb UV and change color. Therefore paintings, furnishings and fabrics should protect from UV to prevent color change or loss. The studies of UV/Vis spectra of various phenanthrene derivatives were conducted using density functional theory (DFT), Hartree-Fork (HF) and Zindo calculations. The ground and excited state configuration has obtained at the TD-B3LYP/32-1G CAMB3PW91/6-31G, and Zindo 3-21G*methods respectively. The absorption spectra for phenanthrene derivatives gave satisfactory results by DFT method in both peak position and intensity. The results reported given by DFT provided a useful insight information of electronic properties of these compounds and it is very important tool for designing and developing the superior UV absorber compounds.

5.17 Benchmark Studies of UV/Vis Spectra of Coumarin Derivatives from Medicinal Plants

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Computational chemistry got a great attention in numerous fields due to its various aspects like easy processing about molecular design, low cost and time saving capabilities. It uses computer simulation to assist in solving chemical problems. Its gives the better theoretical route of synthesizing any compound which gives better yield with low energy path. Coumarin is of much industrial importance due to its use in certain perfumes and fabric conditioners. Coumarin has been used as a gain medium in some dye lasers and as a sensitizer in older photovoltaic technologies and shows absorption of light in UV/Vis region. UV radiation is the most important environmental risk factor for the development of non-melanoma skin cancer, burns the skin and speeds up the aging of skin. Many pigments and dyes absorb UV and change colour. Therefore fabrics, furnishings and paintings need protection from UV to prevent colour change or loss. UV radiations from the Sun are needed by our bodies to produce vitamin D and used in the treatment of skin conditions such as psoriasis. Benchmark studies of UV/Vis spectra of various coumarin derivatives were conducted using density functional theory (DFT), time-dependent DFT (TDDFT) and Zindo calculations. The ground and excited state geometries were obtained at the CAMB3LYP/6-31G+(d,p), TD-B3LYP/6-31G+(d,p) and Zindo 3-21G*(d,p) methods respectively. The characteristics of the absorption spectra for coumarin derivatives were satisfactorily reproduced by TDDFT method in both peak position and intensity. The results reported by TDDFT investigation provide a useful insight into the detailed information on the electronic properties of these compounds and a useful tool for designing and developing the superior UV absorber compounds.

5.18 Fabrication of Flower-Like Hydrozincite by Hydrothermal Method and Its Applications in Adsorption

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The flower-like hydrozincite ($Zn_5(CO_3)_2(OH)_6$) assembled by micro-flakes are synthesized by adopting simple hydrothermal method. Zinc nitrate hexahydrate and urea were used as start materials for the fabrication process. The composition, morphology and structure of the product were characterized by X-ray powder diffraction, scanning electron microscopy and transmission electron microscopy. The instrumental analysis showed that the prepared $Zn_5(CO_3)_2(OH)_6$is an aggregation of micro-flakes, and was polycrystalline with single-crystalline local areas. In order to investigate the adsorption properties of the as synthesized hydrozincite, the residual concentrations of Pb^{2+} in the adsorption experiment were detected by inductive coupled plasma mass emission spectrometry. It worked well under extremely mild conditions, for instance, at room temperature and even in litmus less solution. The prepared hydrozincite ($Zn_5(CO_3)_2(OH)_6$) is an efficient and effective sorbent for Pb^{2+}, It is expected that the prepared hydrozincite will remove a series of heavy metal ions, such as Cu2+, Cd^{2+}.

5.19 ZnO nano-Flowers as Efficient Anti-Bacterial Agent

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Nano ZnO was fabricated by controlled precipitation method in aqueous medium following the acidic route. Characterization has been carried out by Atomic Force Microscopy (AFM), Energy dispersive x-ray analysis (EDX), Fourier transform infra-red spectroscopy (FTIR), Zeta particle size analyzer, scanning electron microscopy (SEM) and X-ray diffraction analysis (XRD). It was found that the hierarchical structure of synthesized nanoscale ZnO was mono-modal nano flowers. The nano ZnO powder obtained was applied on untreated cotton fabric using padding and curing method. The confirmation of deposition of ZnO nano flowers on fabric was done by SEM and AFM. The fabric coated with as- synthesized ZnO nano flowers were used to determine their anti-bacterial effect using shake flask method. Different bacterial strains were used to study the antibacterial effect using Mueller-Hinton medium. The parameters studied were amount of the nano material deposited on the fabric, time of exposure to UV and visible radiations, number of washings of fabric that can adhere coatings and exhibit antibacterial effect, pH of the medium and temperature of surroundings. Evaluation of antibacterial effect was done quantitatively by colony counting, measurement of inhibition zone and images obtained from Confocal laser microscopy (CFLM).Minimum inhibition zone concentration (MIC) was also determined.

5.20 Ambient Ozone Pollution Impact on Biochemical Characteristics of *Vigna radiata* L. Plants by Using Ethylenediurea

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Increase in concentrations of tropospheric ozone (O_3) is one of the main factors affecting world agriculture production. Tropical countries including Pakistan are at greater risk due to their meteorological conditions (high solar radiation and temperature) being conducive to the formation of O_3. A field study was conducted to evaluate the suitability of ethylene diurea (N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N-phenylurea; EDU) in assessing the impact of O_3 on mung bean plants (*Vigna radiata* L.) grown in suburban area of Lahore city situated in a dry tropical region of Pakistan. EDU is a synthetic chemical having antiozonant property. Mean monthly O_3 concentration varied between 79.4 ppb and 105.2 ppb during the experimental period. In comparison to EDU-treated plants, non-EDU-treated plants showed substantial reductions in plant growth and yield under ambient conditions. Significant favorable effects of EDU-application were observed with respect to photosynthetic pigments, soluble protein, ascorbic acid and phenol contents. EDU-treated plants sustained higher levels of pigments, protein and ascorbic acid in foliage as compared to non-EDU-treated ones. The study clearly demonstrated that EDU alleviates the unfavorable effects of O_3 on mungbean plants, and therefore can be used as a tool to assess the growth and yield losses in areas having higher O_3 concentrations.

5.21 Extraction of phenolic compounds from *Cannabis sativa* using Ionic liquids as extraction solvents

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Accretion of human nutrition with high amounts of vegetables, fruits and herbs is highly recommended, due to their beneficial antioxidant activity and free radical scavenging capability. Phenolic compounds are naturally occurring antioxidants usually constituted by fruits and vegetables. In this work, ionic liquids based extraction procedure was successfully applied to extract natural phenolic compounds from Hemp (cannabis sativa). For this purpose, imidazolium based ionic liquids (1-butyl-3-methylimidazolium chloride [BMIM] Cl and 1-butyl-3-methylimidazolium tetraflouroboarte [BMIM] BF_4) were synthesized by using a simple household microwave oven under solvent free conditions, which required a simple exposure of admixed 1-methylimidazole and alkyl halides to microwave irradiation. The synthesized ionic liquids were characterized by using FTIR and NMR spectroscopic techniques. The targeted analytes were extracted using IL-based Microwave and Ultrasound assisted extraction techniques while maceration was selected as a conventional reference extraction method to check the potency of the proposed method. The ionic liquid based extraction procedure was also optimized on certain experimental parameters such as a solid - liquid ratio, extraction time, temperature and, sample particle size. The separation and quantification of the extracted analytes were done by HPLC. Based on the experimental results, the ionic liquid based extraction procedure in conjunction with non-conventional activation methods provided a rapid, efficient and obviously reduced extraction time approach.

5.22 Detection of Formalin in fresh and pasteurized milk

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Formalin is 37% aqueous solution of formaldehyde which is used for various purposes such as solvent, preservatives, reducer etc. It decreases microbial growth in milk by increasing pH and lactose as well as decreasing the lactic acid concentration in the milk. Its residues in food were proscribed because of its carcinogenicity. Several food processing industries abundantly use formalin to enhance the shelf life of food, especially of milk. Keeping in view the toxicity of formalin, present research work has been devised to analyze and compare formalin in fresh and pasteurized milk qualitatively as well as quantitatively. Several chemical methods were used for qualitative determination of formalin using "Ring Test", "Fehling's Test", "Rimini's Phenyl Hydrazine Test" and "Hexamethylene Tetra-amine Test". Quantitative analysis for formalin in milk was done spectrophotometrically using "Nash Reagent" for fresh and pasteurized milk samples. It was observed that the quantity of formalin in fresh milk ranged from zero ppm (Malkhanwala, Dassua, Char Chak, Maquana, Guttwala) to 4.20 ppm (Diglus pura), while in pasteurized milk ranged from 1 ppm (Sample A) to 3.4 ppm (Sample D). According to world health organization, permissible limit of formalin in milk is 1ppm. Results revealed that 79% samples of pasteurized milk contained formalin above the permissible limit. Fresh milk samples collected from urban areas were also observed to contain considerable amount of formalin above permissible limit. Whereas milk samples collected from rural areas contained formalin, upto zero ppm which is in permissible limit.

POSTER PRESENTATIONS

TRACK 5

5.1-P Assessment of knowledge, Behavior and Primary prevention of sun light exposure among students of Bahauddin Zakaria University Multan.

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There has been a significant affects of sunlight exposure on skin disease including cancer. Although no single method is reliable to find parameters for all the disorders due to sun exposure. It is therefore necessary to find out methods for enhancement of our knowledge about drastic effects of sunlight exposure to get preventive measures about skin care. The purpose of this study was to raise sun health awareness levels among university students. Total 400 students were included in this analysis. A questionnaire form was used to evaluate knowledge attitude and behavior among the survey samples. The average level of knowledge concerning the effect of sunlight was found to be 55±5% among students but only 50±4% of men and 33.33±6% of women uses sunscreen in their daily practice. All socio-demographic features were studied, the awareness among people is necessary to change their behavior towards sunlight to protect them from drastic effect of sunlight.

5.2-P Biosorption of Cr (VI) metal ions by chemically treated *Zea maiz* (corn cob)

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In the present study an investigation for the biosorption of Cr (VI) from aqueous solutions using chemically modified *Zea Maize* (corn) cob was conducted. The effect of different parameters such as biosorbant size, biosorbant dose, time, initial metal concentration and pH required for establishment of equilibrium on the biosorption of Cr (VI) ion using chemically modified *Zea Maize* (corn) cob was studied. The Cr(VI) concentration before and after biosorption process was analyzed by atomic absorption spectrophotometer (AAS). The data obtained was analyzed using pseudo first and pseudo second order, Freundlich isotherm and Langmuir isotherm, models. Finally, results were analyzed statistically.

5.3-P Synthesis and Characterization of Azo Dye Derived from Pentaerythritol Derivative

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Approximately 4500 colorants are being used commercially; over 50% belong to the azo dyes, even though newer colorants are being introduced increasingly with time. New synthetic dyes are being introduced in order to meet the persistent need of industry with a wide range of color having improved fastness properties that should be eco-friendly. The present study has been started keeping in view industrial and environmental challenges. In this study azo dye was synthesized by the diazo-coupling reaction of the sulphanilic acid with pentaerythritol derivative, aromatic substituted pentaerythritol derivative has been used as coupling agent. Pentaerythritol derivative has been prepared by the aldol condensation and cross canizzaro reaction. Characterization of synthesized dye has been performed by UV-Vis and FT-IR spectroscopic techniques. Eco-friendly nature of synthesized dye has been evaluated by both photodegradation and its application on fabric. The results were evaluated statistically through the mean ± standard error of mean.

5.4-P Biosorption of Lead (II) from aqeous solutions using *Musa paradisiaca* leaves waste biomass

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Protection of Environment requires the use of natural products instead of chemicals to minimize pollution; this investigation studied the use of waste plant material as naturally occurring biosorbent for the removal of Pb (II) in aqueous solution. Effect of different experimental parameters such as metal concentration, kinetic and pH was studied on the uptake of lead by chemically and physically pretreated *Musa paradisiaca* leaves biomass. The metal ion before and after biosorption processes were analyzed by atomic absorption spectrophotometer (AAS), results obtained fitted well. The adsorption processes were controlled by chemisorptions. FTIR spectroscopic analysis was also carried out on *M.paradisiaca* leaves biomass. The potential applicability of banana leaves based biosorbent could be further examined in a large-scale.

5.5-P Microwave Assisted Study for the Desulphurization of Coal

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Coal is the major source of energy in the world regions where it is abundantly present. An environmental issue associated with it is the emission of sulfur during combustion which causes air pollution. The risk regarding formation of toxic residues in the environment may be reduced if coal is desulphurized before combustion. Present study has been conducted to estimate total sulfur contents by proximate analysis and the sulphur removal from the coal. Three samples of coal (A, B and C) were collected from different mines of District Khushab, Pakistan. Sample B was observed to have higher sulphur contents and calorific value in comparison to A and C. Desulphurization of coal was carried out using microwave and photo-catalytic treatments. Coal samples were ground to different particle sizes followed by microwave irradiation in the presence of KOH medium. In case of photo catalytic oxidation treatment, slurries of coal were prepared in aqueous media and nano-catalyst (ZnO) and H_2O_2 was loaded in each sample and finally irradiated using UV radiation source having power 180-720 watt. In both treatments, the amount of sulfur removed was estimated by precipitating with barium chloride. Response Surface Methodology (RSM) was employed to study operational parameters like particle size of coal, dose of catalyst, time of irradiation, concentration of hydrogen peroxide and power of microwave used. Microwave assisted desulphurization resulted in 60% reduction in sulphur contents whereas in case of advanced oxidation process a decrease of 45% sulphur contents in coal was observed. Hence, microwave assisted desulphurization of coal was revealed to be a good tool for sulpur reduction.

5.6-P Catalytic Reduction of 4-Nitrophenol using Differentially Crosslinked Silver-Poly(N-isopropylacrylamide-co-acrylic acid) Hybrid Microgels

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2, 4, 6 and 8 mol percentage of N,N-methylene-bis-acrylamide (BIS) crosslinker was used to synthesize series of poly(N-isopropylacrylamide-co-acrylic acid) microgels. All samples of microgels were synthesized by precipitation polymerization in aqueous medium. Synthesized microgels were characterized by FTIR and DLS. DLS studies showed that size of crosslinked core was increased with increase in feed content of BIS crosslinker. Later silver (Ag) nanoparticles was synthesized by chemical reduction method using microgels samples as template. Hybrid microgels was characterized by UV-Visible spectroscopy and TEM. TEM images showed that Ag nanoparticles are fabricated within sieves in hybrid microgels. UV-Visible spectroscopic analysis indicated that size and size distribution of nanoparticles was decreased with increase in feed content of crosslinker. Hence results of UV-Visible spectroscopy and DLS support each other. All the hybrid microgels were used as catalyst for reduction of 4-nitrophenol, pollutant. Catalytic reduction was monitored by UV-Visible spectrophotometry. The value of rate constant was decreased from 9.4×10^{-3} to 5.2×10^{-3} s^{-1} with increase in crosslinker feed percentage from 2 to 8. This is due to the hindrance caused by dense network against diffusion of nitrophenol. Catalyst microgels was re-used also. It was observed that catalytic reduction of pollutant remains same almost upto 4 consecutive cycles.

5.7-P Evaluation of effect of chemically treated *Dalbergia sissoo* leaves on biosorption of Pb (II) from industrial waste water

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An investigation for the biosorption of Pb (II) from aqueous solutions using *Dalbergia sissoo* leaves was conducted in the recent study. The effect of various parameters such as biosorbant size, biosorbant dose, initial metal concentration, pH and time required for the equilibrium establishment on the biosorption of Pb (II) ion using *Dalbergia sissoo* leaves was studied. The concentration of Pb (II) before and after biosorption procedure was analyzed by atomic absorption spectrophotometer (AAS). The data obtained was analyzed using Freundlich Langmuir, isotherm, pseudo first and pseudo second order models. After the that the biosorbent will be applied to industrial effluents to remove Cr (VI). Finally, results were analyzed statistically.

5.8-P Synthesis of azo reactive dyes and their application on cotton fabric

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A dye is a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution, and requires a mordant to improve the fastness of the dye on the fabric. Reactive dyes comprise a special class of compounds for the dying of cellulosic fabric. Azo dyes have been established as a major group for the fixation of dye with cellulose. In this study azo reactive dyes were synthesized and then applied on the treated and untreated cotton fabrics using UV-radiation and the applications of high energy radiations in textile. These dyes were applied on the cotton fabric by using laboratory scale exhaust dyeing method. Different dying parameters like salt concentration, temperature, dying time and the pH were optimized. Color strength of the dyed fabrics was measured by using spectrophotometer Spectraflash (SF 600) at Noor Fatima Textile Faisalabad. Fastness properties of these dyes, such as light, washing and crocking were estimated as well. All fastness results revealed that monoazo dyes exhibited satisfactory properties from application point of view. Mercerization along with UV radiation treatment resulted in greater uptake of dye, imparting excellent fastness properties as well, in comparison to the fabric with only bleaching.

5.9-P Effect of microvawe radiation on natural dyeing of cotton from pomegranate rind (*Punica grantum L.*) and turmeric rhizome (*Curcuma longa L.*)

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Recently, natural dyes have been used because these are environmental friendly, less lethal and do not have any detrimental effect on health. Present study has been concerned with dyeing of cotton fabric using mixture of coloring material from pomegranate (Punicagrantum L.) rind and turmeric (Curcuma longa L.) rhizome. For extraction of maximum colorants, the mixture has been treated with microwave radiation at constant power. Pomegranate and Turmeric contain natural colorants grananatonine and curcumin respectively. New shades have been developed by mixing extracts of the two sources. The mixed extracts has been irradiated using microwave radiation source for 0-5 minutes. Different extraction media such as water, methanol and n-hexanol have been used. The dyeing variables such as temperature (35^{o}-75^{o}C) pH (4-10) time (20-80 minutes) and M: L (material to liquid ratio) have been optimized. Fastness properties such as color fastness to light, washing and rubbing have been studied according to AATCC (American Association of Textile chemist and colorist).

5.10-P Arylation Of Protected And Unprotected Anilies Via Pd(0) Catalyzed Suzuki Coupling Reaction And Their Biological Evaluation

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A new series of aniline derivatives was synthesized through Palladium (0) Suzuki cross coupling reaction. Significant yield of protected aniline derivatives was obtained (50-60 %). While unprotected aniline derivatives did not show any yield. The synthesized compounds were screened for antioxidant activity. All the tested compounds showed significant DPPH radical scavenging. It is concluded that compounds could be a potential source of antioxidant agents.

5.11-P Preparation And Physicochemical Characterization Of Microemulsion For Improved Drug Delivery

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Microemulsion system including vegetable oils and bio emulsifiers are the dynamic tool to increase the efficacy and target oriented release of drug. To improve the bioavailability, targeted release and loading capacity of oral anti-lipidimic drugs Rosuvastatin and Atorvastatin, new oil in water (o/w) microemulsion systems were formulated. These systems included olive oil, tween 80/60, glycerin and phosphate buffer. Drugs were loaded in each selected microemulsion 5% by weight of weight fraction of oil. Conductivity, viscosity, surface tension, density and pH of the microemulsion system were studied. The phase behavior, conductivity, viscometry, tensiometry and pH behavior showed that gradual transforms occurring in the microstructure of microemulsion. Microemulsion loaded drug prevent toxicity and irritation. The loaded microemulsion increases the stability, bioavailability and activness of the drug. Stability of the drug in microemulsion was analyzed by centrifugation. Results showed that all the microemulsion formulations were single phase, optically transparent and remained stable for the period of 4-5 months.

5.12-P ^{1}H-NMR Study Of New Formulated O/W Microemulsions For Chloramphenicol

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Microemulsions are optically clear, homogeneous and thermodynamically stable system to increase the solubility, bioavailability of drug and able to entrap the drug for a prolong time. Two new systems of oil in water MEs for chloramphenicol were formulated by using isopropyl myristate and oleic acid, Tween 60/20 with short chain alcohols and phosphate buffer (pH=7). The different parameters of formulated MEs such as phase behavior, pH, refractive index, conductivity, surface tension and viscosity were studied. The location of drug in formulations was investigated by ^{1}H-NMR spectroscopy. It was revealed by ^{1}H-NMR study that chloramphenicol molecules should be trapped in the hydrophilic shells of ME, which were consisted of many oxyethylene groups. This study implied that the drug was screened from bulk water and its stability was increased. The characterizations suggested that the formulated microemulsions were optically clear and stable and can be used as drug carrier in ocular drug delivery.

5.13-P Isothermal and thermodynamical studies of Congo Red dye biosorption on Formalin-Treated *Citrillus lanatus* (Melon) Husk

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The adsorption characteristics of Congo Redon formaldehyde-treated *Citrillus lanatus* (Melon) melon husk was investigated on batch scale. Characteristic functional groups were identified by FT-IR and change in surface morphology was checked by taking SEM images. Congo Reddye solution adsorption equilibrium was attained after 45 minutes. Isothermal studiesshown that the Langmuir model was followed more indicating monolayer biosorption and maximum removal capcity was 34.47 mg/g.Kinetic studies shown that biosorption data was well fitted on pseudo second-order model. ΔG^o, $\Delta H^o$and ΔS^o(thermodynamic parameters) were negative, indicating that the biosorption mechanism was exothermic.

5.14-P Estimation of shelf-life of natural colorants extracted from eucalyptus bark and pomegranate peel

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The demand of natural colorants is increasing globally because of their medical and therapeutic properties and also due to the recognized harmful effects of synthetic colors. The present research work was aimed to estimate the shelf-life of natural colorants extracted from eucalyptus bark and pomegranate peel. Furthermore the extract of eucalyptus bark and pomegranate peel was assessed for their anti-oxidant activities, total phenolic, and their anti-microbial potential at various pH. The degradation of both dyes was observed with the passage of time to estimate the effect of environmental factors on it. Pomegranate peel showed good anti-oxidant and anti-microbial activity as compared to eucalyptus bark. Pomegranate peel extract has inhibition zone 20.06mm and eucalyptus bark extract has 12.83mm. Eucalyptus showed good color strength having K/S=6.23 as compared to pomegranate peel.

5.15-P Effect of UV treatments on the Color Quality of printed Cotton Fabrics

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The different combinations of Pigment Yellow 12 & Pigment black 7 has been selected for study. The cotton fabric and dye solution were irradiated to UV radiation for different times. Printing was performed using un-irradiated and irradiated cotton with un-irradiated and irradiated mixed powder of Pigment yellow 12 & pigment black 7. The extracts were obtained by using combinations of different concentrations of irradiated mixed pigments powder. In order to improve color fastness properties, the pigment powder and fabric were exposed to UV radiation. The printed samples are subjected to a CIE Lab system with a spectra flash spectrophotometer (SF650) for the evaluation of L* (lighter/darker), a* (redder/greener) and b* (yellower/bluer) values. Finally, ISO standard methods were employed to study color fastness to light, washing and rubbing (dry and wet) in order to observe the influence of UV radiation on the printing behavior of combinations of pigment powder. It was observed that UV radiation not only enhances the color strength of dye on irradiated cotton fabric using low concentrations of dye, but also improved the color fastness properties of pre-irradiated cotton fabric by using pre-irradiated mixed pigment powder.

5.16-P Effective exclusion of Pb (II) from aqueous solution by magnetic nanoparticle (Fe_3O_4) impregnated onto rice bran

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The discharge of toxic heavy metals into the aquatic environment via physico-chemical and industrial procedures were found to be dangerous for the survival of living beings. The purpose of present study was to examine the adsorption potential of NaOH treated rice bran impregnated with Fe_3O_4 magnetic nanoparticles (SH-MNP) for the efficient removal of Pb (II) metal from aqueous media. Effects of various experimental parameters like pH, time and initial metal concentration were optimized. The maximum adsorption of Pb (II) was observed at pH 4.0 with 0.01g/100mL biosorbent dose. The outcomes showed that SH-MNP had high adsorption potential i.e. 85.4% for Pb (II). Application of equilibrium and kinetic models suggested that Pseudo-second-order kinetic and Freundlich adsorption isotherm model illustrated better fitness to the experimental data. The adsorptive properties of SH-MNP were characterized by SEM/EDX and FTIR analysis. FTIR analysis showed the involvement of carbonyl, hydroxyl, carboxyl, amino and Fe-O groups in the sorption process. The results revealed that magnetic nanocomposites of rice bran have high metal ion uptake capacity and could be successfully used for wastewater treatment.

5.17- P Chemical Characterization And Bilogical Activities Of *Mentha arvensis* Essential Oil

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Mentha arvensis is an important aromatic herb of tropical and subtropical climate. The essential oil of *Mentha arvensis* commonly known as mint or menthol oil has been used widely in the pharmaceuticals, food and flavor industry mainly due to its active ingredient, L-menthol known for typical refreshing note. The purpose of present study was to study the chemical compositoin of the essential oil of *Mentha arvensis* grown in the region of Faisalabad and to evaluate its antifungal and antioxidant activity. For this purpose the aerial parts of *Mentha arvensis* were subjected to steam distillation to extract mint oil. GC/MS analysis of this oil showed that (-)-Menthol, Menthone, Isomenthol, Limonene, Pulegone, and Caryophyllene were major components. The antioxidant activity of oil was evaluated by DPPH scavenging activity and TPC. The results showed that the essential oil of *Mentha arvensis* has a strong antioxidant potential. The oil also showed good antifungal activity against *Penicillium oxalicum, Aspergilus niger* and *Pycnoporus sanguineus*. This research study suggests that *Mentha arvensis* grown in the region of Faisalabad has good quality of essential oil with good percentage of important chemical components and high biological activities and hence can be useful for herbal medicinal companies.

5.18- P Plant Extract Mediated Synthesis Of Silver Nanoparticles

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The development of nanoparticles ranges from 1-100nm, for the delivery of therapeutic agents has introduced new opportunities for the improvement of medical treatment. In the present study, an eco-friendly process for rapid synthesis of silver nanoparticles has been reported. The synthesis of silver nanoparticles was done by using *Citrus reticulate* fresh peel extract as bioreductant which exhibited strong potential for rapid reduction of silver ions with nearly the constant size of particles (40nm). This indicated that fresh peel extract may have good reducing and capping agents like polyols, phenols, esters and ethers which may involved in the synthesis and stability of AgNPs. The varying concentrations of $AgNO_3$ (0.5 to 3 mM) were also used to find the optimum value. The reaction process was simple and convenient to handle, and was monitored by visual analysis and by using UV-Vis spectroscopy. The medicinal value of *Citrus reticulate* and Ag is already established so the combination of *Citrus reticulate* fruit extract and its mediated nanoparticles can be effectively used in drug delivery system to cure different diseases.

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