



2nd INTERNATIONAL CONFERENCE OF BIOCHEMISTRY, BIOTECHNOLOGY and BIOMATERIALS



ICBBB-2018

December 09-11, 2018

Abstract Book



**Biochemistry for better health and
environment in the developing world**



Organized by

*Department of Biochemistry
University of Agriculture, Faisalabad - Pakistan*



2nd International Conference of Biochemistry, Biotechnology and Biomaterials

(ICBBB-2018)

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Conference Theme

**Biochemistry for better health and environment
in the developing world**

**Abstract Book Compiled by
Professor Dr Tahira Iqbal (Convener, Scientific Committee)**

**Organized by Department of Biochemistry
University of Agriculture
Faisalabad, Pakistan**



2nd International Conference of Biochemistry, Biotechnology and Biomaterials (ICBBB-2018)

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Biochemistry for better health and environment in the developing world

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Professor Paul C. Engel, Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland

Plenary 2: Genome wide quantitative trait loci reveal the genetic basis of cotton fiber quality and yield related traits in G.hirsutum RIL population

Professor Youlu Yuan : ¹Key Laboratory of Biological and Genetic Breeding of Cotton, The Ministry of Agriculture; Institute of Cotton Research, Chinese Academy of Agricultural Sciences; Anyang, Henan, 455000 China

²Research Base, Anyang Institute of Technology, State Key Laboratory of Cotton Biology, Anyang, Henan, 455000 China

Plenary 3: Combined Linkage and Association Mapping of Fiber Quality Traits and Yield Components in Upland Cotton Using Enriched High-Density SNP Markers

Professor Wankui Gong: State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, 455000 Anyang, Henan, China,

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Professor Muhammad Mukhtar: Faculty of Industrial Sciences & Technology, University of Malaysia Pahang 26300 Gambang, Pahang, Malaysia

Plenary 5: Mushrooms biomaterial for promoting health in human and animals

Omon. S. Isikhuemhen : Mushroom Biology & Fungal Biotechnology Laboratory, NRED, CAES, North Carolina A&T State University, Greensboro, NC 27411, USA.

Email: omon@ncat.edu

Plenary 6: Bioactivity of natural antioxidants

Prof. Dr. Ahmed Hassan El-Ghorab: Department of Chemistry, Faculty of Science, Jouf University, Saudi Arabia KSA.

Plenary 7: Mechanism of the formation of high mannose n-glycans in advanced cancer

Pi-Wan Cheng: Department of Biochemistry and Molecular Biology, College of Medicine, and Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, USA

Plenary 8: Genome-wide characterization of mutations induced by genetic carcinogens using next-generation sequencing

Tao Chen: Division of genetic and molecular toxicology, national center for toxicological research, food and Drug Administration, Jefferson, AR, 72079

Plenary 9: “Clickable” siRNA-polyisoprenoids-conjugated nanoparticles: a new pharmacological approach for cancers harboring fusion oncogenes

M. Caillaud¹, B. Parola², H.M. Ali¹, C. Gracia¹, B. Stella², S. Arpicco², D. Desmaële³, P. Couvreur³, L. Massaad-Massade¹, G. Urbinati¹

¹Vectorology and Anticancer Therapies, UMR 8203 CNRS, Univ. Paris-Sud, Gustave Roussy, Université Paris-Saclay, 94805 Villejuif, France. Electronic address: giorgia.urbinati@gustaveroussy.fr.

²Department of Science and Pharmaceutical Technology, University of Turin, 10125 Turin, Italy.

³Institut Galien, UMR 8612 CNRS, Université Paris-Saclay, Faculty of Pharmacy, 92296 Châtenay- Malabry, France.

PLENARY 1: ENZYME KINETICS IN THE DEVELOPMENT AND ASSESSMENT OF ENGINEERED ENZYMES FOR AMINO ACID ANALYSIS OR SYNTHESIS

Paul C. Engel

Conway Institute of Biomolecular and Biomedical Research,
University College Dublin, Dublin, Ireland

Over the past 35 years it has become increasingly easy to alter the function of natural enzymes by mutagenesis. This may be either site-directed mutagenesis guided by structural insight or random mutagenesis followed by screening to select favourable mutations. All too often the assessment of the mutants is based exclusively on a standard assay under optimal conditions. This presentation will make the case for more thorough kinetic scrutiny and will offer a number of examples in which detailed kinetics has been essential in judging what has or has not been achieved. These examples will be drawn from work in the author's research group on amino acid dehydrogenases and aminotransferases aimed at producing effective catalysts either for diagnostic analysis or for chiral synthesis. In the first case it is important a) to have a high degree of specificity and also high levels of activity at low substrate concentrations. In the second case. In the second case relevant specificity is of course also important, but broad specificity is now an advantage in engineering a versatile catalyst applicable to a range of related feedstock substrates. Kinetics can still offer guidance with regard to what properties can realistically be tackled as targets for improvement.

PLENARY 2: GENOME WIDE QUANTITATIVE TRAIT LOCI REVEAL THE GENETIC BASIS OF COTTON FIBER QUALITY AND YIELD RELATED TRAITS IN *G.HIRSUTUM* RIL POPULATION

Zhen Zhang^{1,2†}, Junwen Li^{1,2†}, Muhammad Jamshed^{1†}, Yuzhen Shi^{1†}, Aiyong Liu¹, Juwu Gong¹, Shufang Wang¹, Jianhong Zhang¹, Fuding Sun¹, Fei Jia¹, Qun Ge¹, Liqiang Fan¹, Zhibin Zhang¹, Jingtao Pan¹, Min Zhang¹, Senmiao Fan¹, Yanling Wang¹, Quanwei Lu², Ruixian Liu¹, Xiaoying Deng¹, Xianyan Zou¹, Xiao Jiang¹, Ping Liu¹, Pengtao Li², Muhammad Sajid Iqbal¹, Jinping Hua³, Chuanyun Zang⁴, Juan Zou⁴, Hong Chen⁵, Qin Tian⁵, Xinhe Jia⁶, Baoqin Wang⁶, nijiang Ai⁷, Guoli Feng⁷, Yumei Wang⁸, Mei Hong⁹, Shilin LI⁹, Wenming Lian¹⁰, Bo Wu¹⁰, Chaojun Zhang¹, Haihong Shang^{1,2*}, Wankui Gong^{1,2*} and **Youlu Yuan^{1,2*}**

¹Key Laboratory of Biological and Genetic Breeding of Cotton, The Ministry of Agriculture; Institute of Cotton Research, Chinese Academy of Agricultural Sciences;

Anyang, Henan, 455000 China

²Research Base, Anyang Institute of Technology, State Key Laboratory of Cotton Biology, Anyang, Henan, 455000 China

³College of Agronomy and Biotechnology, Key Laboratory of Crop Heterosis and Utilization of Ministry of Education, Beijing Key Laboratory of Crop Genetic improvement, China Agricultural University, Beijing 100193, China

⁴Shandong Cotton Research Center, Jinan, 250100, China

⁵Cotton research Institute, Xinjiang Academy of Agricultural and Reclamation Science, 832000, China

⁶Zhengzhou Research Institute of Agricultural and Forestry Sciences, Zhengzhou 450000, China

⁷Shihezi Agricultural Science & Technology Research Center, Shihezi 832000, China

⁸Institute of Cash Crops, Hubei Academy of Agricultural Science, Wuhan, 430064 China

⁹Institute of Agriculture, Bayingolin Mongol Autonomous Prefecture, Korla, Xinjiang 841000, China

¹⁰Institute of Agricultural Science and Technology, Agricultural Production Division 1, Xinjiang Production and Construction Corps, Alar Xinjiang 843300, China

†: These authors make the same contributions

Corresponding authors: Youlu Yuan (yuanyoulu@caas.cn) or Wankui Gong (wkgong@aliyun.com) or Haihong Shang (shanghaihong@caas.cn)

Cotton was widely planted in the world. To identify QTL/Gene for fiber quality and yield, a RIL population was developed between upland cotton. A consensus genetic map covered the whole upland cotton genome was constructed by three types of marker (8295 markers, 5197.17cM). Combined with the 17 environments phenotype data, 983 QTLs were identified for fiber quality and yield traits 198 of them were stable, mainly distributed on chromosome 4, 6, 7, 13, 21, and 25. Thirty-seven QTL clusters were identified, in which 92.8% of two-pair-traits with significantly medium or high positive correlation had the same additive effect direction of QTLs, and all of the two-pair-traits with significantly medium or high negative correlation had the opposite additive effect direction. 1297 genes were discovered in the QTL clusters and 414 of them were expressed in two RNA-seq data Plenty of novel genes were discovered and 20 of them were promising genes. This was the first report about constructing a high coverage and saturation consensus map, and identifying most number of stable QTLs and QTL clusters for fiber quality and yield, elucidating the genetic basis of correlation between fiber quality and yield is related to QTL cluster on the whole genome. It could provide information about the genetic basis of cotton fiber and yield and help cotton breeders to improve the fiber quality and yield synchronously.

Key words: upland cotton, consensus genetic map, fiber quality, fiber yield, QTL clusters, genetic correlation, gene expression level, promising gene

PLENARY 3: COMBINED LINKAGE AND ASSOCIATION MAPPING OF FIBER QUALITY TRAITS AND YIELD COMPONENTS IN UPLAND COTTON USING ENRICHED HIGH-DENSITY SNP MARKERS

Ruixian Liu, Juwu Gong, Xianghui Xiao, Junwen Li, Aiying Liu, Hailong Shang, Yuzhen Shi, Qun Ge, Muhammad S. Iqbal, Xiaoying Deng, Shaoqi Li, Jingtao Pan, Abdul Hafeez, Benna Alam, Youlu Yuan* and **Wankui Gong***

State Key Laboratory of Cotton Biology, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, 455000 Anyang, Henan, China

It is of great importance to identify quantitative trait loci (QTL) controlling fiber quality traits and yield components for future marker-assisted selection (MAS) and candidate gene function identifications. In this study, two kinds of traits in 231 F 6:8 recombinant inbred lines (RILs), derived from an intraspecific cross between Xinluzao24, a cultivar with elite fiber quality, and Lumianyan28, a cultivar with wide adaptability and high yield potential, were measured in nine environments. This RIL population was genotyped by 122 SSR and 4729 SNP markers, which were also used to construct the genetic map. The map covered 2477.99 cM of *Gossypium hirsutum* genome, with an average marker interval of 0.51 cM between adjacent markers. As a result, a total of 134 QTLs for fiber quality traits and 122 QTLs for yield components were detected, with 2.18–24.45 and 1.68–28.27% proportions of the phenotypic variance explained by each QTL, respectively. Among these QTLs, 57 were detected in at least two environments, named stable QTLs. A total of 209 and 139 quantitative trait nucleotides (QTNs) were associated with fiber quality traits and yield components by four multilocus genome-wide association studies methods, respectively. Among these QTNs, 74 were detected by at least two algorithms or in two environments. The candidate genes harbored by 57 stable QTLs were compared with the ones associated with QTN, and 35 common candidate genes were found. Among these common candidate genes, four were possibly “pleiotropic.” This study provided important information for MAS and candidate gene functional studies.

Keywords: upland cotton, QTL, multilocus GWAS, QTN, candidate gene, fiber quality traits, yield components

PLENARY 4: CELL CULTURE MODELS – FROM A SINGLE CELL TO AN ORGAN CULTURE

Muhammad Mukhtar

Faculty of Industrial Sciences & Technology

Universiti Malaysia Pahang

26300 Gambang, Pahang, Malaysia

Cell culture models are conventionally used for basic and applied biomedical research. Generally two-dimensional (2-D) *in vitro* cell culture setups have been used to observe the effects of various chemicals including anticancer drug discovery and transport of biomolecules through a monolayer of adherent cells. Our laboratory developed a 2-D cell culture model for the human brain in which four different types of cells can interact with each other. Among these, the brain microvascular endothelial cells in conjunction with astrocytes form a functional blood-brain barrier, whereas neurons at the bottom layer of two compartment system can communicate with all the cellular elements including monocytes/macrophages in the top layer. Recent developments in cell culture models have led to the establishment of three-dimensional (3-D) cell culture models mimicking *in vivo* environments. These models are not only used for individual or mixed cells culture instead organs culture have also been developed through this unique cell culturing methodology. Several mini organoids created through 3-D models proved their physiological functions like *in vivo*. We have evaluated both the 2-D and 3-D existing cell culture models in their relevance to neurogenerative disorders. This involved critical evaluation of empirical information related to both these models. Our data suggest that a 3-D cell culture model no doubt offers a unique set up closely resembling with the *in vivo* environments as well as cellular interactions. However, several delimitations are proving the utility of 2-D cell culture model in neurodegenerative disease including several others. Comparative evaluations related to 2-D and 3-D cell culture models suggest that 2-D model provides more physiological relevant details about neurodegenerative drug discovery. However, the 3-D cell culture model has better utility in the regeneration of degenerated tissues in the brain.

PLENARY 5: MUSHROOMS BIOMATERIAL FOR PROMOTING HEALTH IN HUMAN AND ANIMALS

Omon. S. Isikhuemhen

Mushroom Biology & Fungal Biotechnology Laboratory, NRED, CAES, North Carolina
A&T State University, Greensboro, NC 27411, USA.

Email: omon@ncat.edu

Though mushrooms have been used as food for centuries all over the world, they have become a source of new biomaterial and compounds useful for health improvement in humans and animals. Some of the popular mushrooms used as health supplements, though are cultivated today, are *Ganoderma spp*, *Lentinula edodes*, *Trametes versicolor*, *Pleurotus spp*, etc. It is an established fact that mushrooms are functional foods with their immense nutraceutical properties, due to their content of bioactive compounds like β - and α -glucans, triterpenoids, antioxidants, glycoproteins, lectins, etc. These compounds have therapeutic and immunomodulation properties. There are products of various types in the market and they have started to reach worldwide sales that amounts to millions of dollars. Though application in human health has been the focus in the past years, their use in farm animal feed is on the rise. The presentation will summarize existing knowledge on the use of mushrooms in human health and focus on recent advances in their use in animal production. Modern scientific approaches have allowed us to exploit mushrooms for food and biomaterials useful for various purposes, without massive depletion through harvesting from the forests. However, many ‘difficult to cultivate’ mushrooms, especially the mycorrhiza used for food and from which many medicinally active compounds are being discovered are still obtained from the forests. While the discovering of such compounds is good, we need massive investment in research and development so that we can use modern techniques now available for the extraction and synthesis where necessary, in order to expand the array of biomaterials from mushrooms to serve mankind. This could become multibillion dollar industry in which those who make the investment now will rule the future with considerably high financial returns.

Keywords: Biomaterials, Food production, Human and animal health, Medicinal mushrooms.

PLENARY 6: BIOACTIVITY OF NATURAL ANTIOXIDANTS

Prof. Dr. Ahmed Hassan El-Ghorab

Department of Chemistry, Faculty of Science, Jouf University, Saudi Arabia KSA

Antioxidants are important in the diet as it is rich in unsaturated fatty acids, which are easily oxidized. Consumers prefer natural antioxidants to synthetic antioxidants, mainly for many reasons. The common daily diet contains about 1 g natural antioxidants even if no natural antioxidants have been added for lipid stabilization. Their main sources are cereals, fruits, vegetables, and beverages. Only a part of the natural antioxidants is absorbed and used as free-radical scavengers in vivo. Natural antioxidants should be added to food in larger amounts in comparison with synthetic antioxidants as they are less active, but the actual activity depends very much on certain conditions and food. so it is very important maximize the value addition of indigenous food by-products as a good source of natural antioxidant and replace high-polyenoic oils in the diet with high-oleic oils, and to use alternative methods of food protection against autoxidation.

Key words: Natural and synthetic antioxidant, indigenous foods, autoxidation

PLENARY 7: MECHANISM OF THE FORMATION OF HIGH MANNOSE N-GLYCANS IN ADVANCED CANCER

Pi-Wan Cheng

Department of Biochemistry and Molecular Biology, College of Medicine, and Buffett Cancer Center, University of Nebraska Medical Center, Omaha, NE, USA

Elevated high mannose N-glycans has been reported in many cancers. But, the mechanism was not clear. Recently, we discovered that Giantin, a *cis*-Golgi type 1 membrane protein, is the primary Golgi targeting site for the vesicles that transport glycosylation enzymes and their substrates from Endoplasmic Reticulum. This Giantin function was lost in aggressive prostate cancer cells due to failure to be phosphorylated and form dimer (Petrosyan et al. Mol Cancer Res 12: 1704, 2014). This defect forces these vesicles to use an alternative site (GM130-GRASP65) in the *cis*-Golgi for Golgi targeting, thus creating a disorganized glycosylation environment at this site. As a result, α -mannosidase IA (Man IA), the enzyme responsible for trimming Man₈GlcNAc₂ down to Man₅GlcNAc₂ to initiate formation of complex-type N-glycans by N-acetylglucosaminyltransferase I, fails to process all high mannose N-glycans to Man₅GlcNAc₂. The Man₆₋₈GlcNAc₂ N-glycans that are not processed at this site cannot be processed further through subsequent glycosylation steps. Further, Man₅GlcNAc₂ that fails to be processed by N-acetylglucosaminyltransferase I also will remain unchanged (Bhat et al: BBA - General Subjects 1861: 2891, 2017). By proximity ligation assay, Man IA was detected at the Giantin site in androgen-dependent prostate cancer cells and stages I & II prostate cancer. However, Man IA was localized at the GM130-GRASP65 site in androgen-refractory prostate cancer cells and stages III & IV prostate cancer. The differential Golgi localization of Man IA may be used for identification of indolent versus advanced prostate cancer cells. To sum it up, altered Golgi targeting of glycosyltransferases and Man IA from Giantin to GM130-GRASP65 as prostate cancer progresses from indolent to advanced stages causes incomplete processing of high mannose N-glycans resulting in their accumulation. This observation forms the basis for developing an assay for identification and a targeted therapy for treatment of advanced prostate cancer.

**PLENARY 8: GENOME-WIDE CHARACTERIZATION OF MUTATIONS
INDUCED BY GENETIC CARCINOGENS USING NEXT-GENERATION
SEQUENCING**

Tao Chen

Division of Genetic and Molecular Toxicology, National Center for Toxicological Research,
Food and Drug Administration, Jefferson, AR, 72079

Mutations are heritable changes in the nucleotide sequence of DNA that can lead to many adverse effects, such as cancers. Genotoxicity assays have been used to identify chemical mutagenicity and carcinogenicity. Current FDA-recommended mutation assays, such as the Ames test and mouse lymphoma assay, predict mutagenicity of test agents in the genes that allow mutant cells to be positively selected when mutations occur in the genes. These assays only detect mutations related to the genes, but not the whole genome. The mutations induced by the test agents may bias to certain types of mutations due to the target genes' natures. Although the assays have been used for many years, a new mutation assay that can directly measure all types of mutations in genome has been expected for a long time. Recently developed next-generation sequencing (NGS) technology allows us to detect genome mutations in the cells directly. In our laboratory, we have used whole genome sequencing method to screen mutagens using *Salmonella typhimurium* TA100 cells, a bacteria system, to detect germline mutations in *Caenorhabditis elegans*, a worm system, and to evaluate mutational spectra in mouse lymphoma cells, a mammalian system. The results show that NGS technology can sensitively detect mutation induction caused by genetic carcinogens and effectively evaluate the different types of mutations including base pair substitutions, insertions and deletions (indels), loss of heterozygosity, and chromosome number changes, suggesting that the unparalleled advantages of NGS for evaluating mutagenicity of chemicals can be applied for the next generation of mutagenicity tests.

PLENARY 9: “CLICKABLE” SIRNA-POLYISOPRENOIDS-CONJUGATED NANOPARTICLES: A NEW PHARMACOLOGICAL APPROACH FOR CANCERS HARBORING FUSION ONCOGENES

M. Caillaud¹, B. Parola², H.M. Ali¹, C. Gracia¹, B. Stella², S. Arpicco², D. Desmaële³, P. Couvreur³, L. Massaad-Massade¹, G. Urbinati¹

¹Vectorology and Anticancer Therapies, UMR 8203 CNRS, Univ. Paris-Sud, Gustave Roussy, Université Paris-Saclay, 94805 Villejuif, France. Electronic address: giorgia.urbinati@gustaveroussy.fr.

²Department of Science and Pharmaceutical Technology, University of Turin, 10125 Turin, Italy.

³Institut Galien, UMR 8612 CNRS, Université Paris-Saclay, Faculty of Pharmacy, 92296 Châtenay-Malabry, France.

Fusion oncogenes are the products of chromosomal rearrangements that are responsible for the development of more than 20% of cancers. Indeed, fusion transcription factors are driver oncogenes in many tumor types, thus, represent crucial targets. As their presence is observed only in cancer cells and only a minority of the known fusion proteins contain a “druggable” domain for small molecules, their targeting *via* small-interfering RNA (siRNA) represents a new therapeutic breakthrough for a personalized therapy. The siRNA, short oligonucleotides able to knockdown genes at mRNA level, are highly specific and efficient at very low doses; however their administration is a major challenge because the biological efficacy is hampered by their poor stability in biological environments. Therefore, to protect their pharmacological activity they require vectorization. To deliver and protect siRNA, we developed a method based on coupling siRNA to polyterpenes taking advantage of the unique property of polyisoprenoid chains to adopt a compact molecular conformation and to self-assemble into nanoparticles (NPs). More particularly, a new chemical strategy was applied to conjugate squalene (SQ) and solanesol (SOLA) to siRNA consisting in an activated variant of the azide-alkyne Huisgen cycloaddition also known as Copper-free click chemistry. We conjugated siRNA against TMPRSS2-ERG, a fusion oncogene found in more than 50% of prostate cancers to SQ or SOLA. The resulting nanoparticle formulations, siRNA TMPRSS2-ERG-SQ NPs and siRNA TMPRSS2-ERG-SOLA NPs were investigated for their biological activity. *In vitro*, both formulations were able to decrease the corresponding oncogene and oncoprotein expression, however, *in vivo*, only the siRNA TMPRSS2-ERG-SQ NPs showed antineoplastic activity that seems to be tightly related to the dissimilar biodistribution behavior of the two formulations. In conclusion, we performed a new approach easily

transposable for pharmaceutical development to synthesize siRNA-SQ and siRNA-SOLA bioconjugates and obtained stable, reproducible and efficient siRNA-nanoparticles. These studies would offer an innovative nanomedicine for patients affected by cancer pathologies harbouring fusion oncogenes. The robustness of the process could be extended to several other polyterpenes and likely applied to other siRNA, thus rendering the clickable” siRNA-polyisoprenoid-conjugated nanoparticles a new delivery platform for oligonucleotides.

TRACK 1

CLINICO-MEDICAL AND NUTRITIONAL

BIOCHEMISTRY, MEDICINAL PLANTS

AND TRADITIONAL MEDICINES

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- 2. DR. NAZISH JAHAN**
- 3. DR. FOZIA ANJUM**
- 4. DR. ABID ALI**

KEYNOTE LECTURES

TRACK 1

**K-1.1. A GREEN APPROACH FOR THE PRODUCTION OF BIODIESEL FROM
FATTY ACIDS OF CORN OIL DEODORIZER DISTILLATE**

Syed Tufail Hussain Sherazi*

National Centre of Excellence in Analytical Chemistry, University of Sindh,
Jamshoro -76080, Pakistan

*Corresponding Author Email: tufail.sherazi@gmail.com

A novel alginic acid derived tin catalyst, tin alginate (Sn–Alg), was successfully synthesized, characterized and applied for methyl esterification. Initially, the amount of catalyst, methanol to fatty acid ratio and reaction time were optimized using an oleic acid standard for esterification. The optimal reaction conditions were found to be 4% catalyst, 1 : 12 oleic acid to methanol mole ratio and 2 h reaction time with 98.7% fatty acid methyl ester recovery. The capability of Sn–Alg beads to esterify the fatty acids of corn deodorizer distillate was evaluated. High recovery (97.6%) of esters was obtained after 8 cycles using reprocessed catalyst under the optimized parameters. The results of the present study indicated that based on the environmental pollution, reusability, avoiding the use of potassium or sodium hydroxides or sulphuric or phosphoric acids, and ease of catalyst separation, the solid Sn–Alg catalyst has a great potential for biodiesel production from highly free fatty acid deodorizer distillates.

**K-1.2. EDIBLE FATS AND OILS: BIOCHEMICAL SIGNATURES AND
SIGNIFICANCE AS DEPICTED THROUGH OPTICS**

Hina Ali, PhD

Principal Scientist

National Institute of lasers and Optronics, Islamabad

Food is essential for sustenance and human nourishment, therefore its quality and authenticity is of major concern and has drawn attentions of stakeholders and consumers. Multichannel fingerprinting techniques are available for the assessment of food quality: a multifaceted issue that involves many aspects. Spectroscopic techniques offer a quick and non-invasive tool to determine the food quality. Fat is one of the major nutrients and is important in modulating disease risks. The comparative study of edible oils, spreads and ghee based on fatty acid composition using Raman spectroscopy. It has a potential to differentiate desi ghee, mono-unsaturated extra virgin olive oil (EVOO) from other processed ghee, spreads and polyunsaturated oils. Desi ghee contains conjugated Linoleic acid (CLA) has proved to be anti-carcinogenic, anti-inflammatory and anti-allergic properties. Raman spectroscopy along with chemometric techniques can potentially be utilized for efficient and reliable compositional analysis and authentication of commercially available edible oils, spreads and ghee.

ORAL PRESENTATIONS

TRACK 1

O.1.1. A REVIEW: “ANTI MICROBIAL ACTIVITY THROUGH ESSENTIAL OILS OF *DRACOCEPHELM* AND *FAGOPYRUM* SPECIES”

Maria Asghar, Arshia Nazir, Adeena Saeed, Kausar Malik*, Husnain Qamar, Anita Shahid, Afifa Saddique, Syed Rizwan Abbas and Adnan Ahmad Qazi .

Centre of Excellence in molecular Biology, University of the Punjab, Lahore.

Corresponding author Email: kausarbasit786@yahoo.com

The constant adaptability and resistance of pathogenic microorganisms to antibiotics led to the search and development of new plant-derived antimicrobial agents that could be used cost effectively in underdeveloped countries. Several plants, shrubs and herbs have been used for the medicinal purposes even at the time of Egyptian civilization (Ebers Papyrus). Numerous compounds such as alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, terpenes and anthraquinones present in the plants, herbs or shrubs possess the antimicrobial capabilities against the pathogenic microorganisms. *Dracocephelum* and *Fagopyrum* (buckwheat) species being found in Central Asia and Northern Pakistan respectively are used as conventional medicines to treat various diseases including wounds healing, headaches, stomach and liver problems as well as allergies. Essential oils of *Dracocephelum* and *Fagopyrum* (buckwheat) sp. prepared by hydrodistillation possess antibacterial activities against both gram positive and gram negative bacteria as they contain monoterpenoids, oxygenated compounds, geranial, neral, geranoil and nonanoic acid, benzothiazole respectively. Besides this, these plants also exhibit antifungal, antioxidant, anticancer and antidiabetic activities. In addition, plant extracts of *Dracocephalum* and *Fagopyrum* sp. have insecticidal activities against red flour beetle and confused flour beetle respectively. Extracts of *Dracocephelum* and *Fagopyrum* (buckwheat) sp. can also be used for the production of silver nanoparticles that already have potential applications in cancer therapy. It is likely that active agents of these plants will be used as FDA approved food additives. Pathways for the production of the active agents of *Dracocephelum* and *Fagopyrum* (buckwheat) sp. have been elucidated due to advancements in sequencing and recombinant DNA technology. In future, metabolic engineering of bacterial strains will be used to increase the yield of natural plant products.

Key words: antimicrobial agents, pathogenic resistance, *Dracocephelum* species, *Fagopyrum* species, essential oils, silver nanoparticles.

O.1.2. INHIBITORY EFFECTS OF FUNGAL RED PIGMENT BY *PENICILLIUM IMRANIANUM* AND ESTROGEN RECEPTOR BETA ON LYMPHOMA GROWTH

Muhammad Sharif Hasni

Institute of Biochemistry, University of Balochistan, Pakistan

Corresponding author Email: sharifhasni@gmail.com

Lymphoma is a heterogeneous group of malignancies derived from the lymphoid system that accounts for roughly 4 % of cancers worldwide. A significant improvement during the last decade was made possible with the introduction of an anti-CD20 monoclonal antibody, rituximab, which has been utilized widely in the treatment of B cell lymphomas leading to significantly better outcomes, which in part have contributed to the reduced mortality rates. In the current study we characterized the red pigment secreted by *Penicillium imranianum* and also screened its biotechnological applications. The biotechnological applications experiments were performed to check its antibacterial, antifungal, anticancerous and antioxidant activities. Treatment with red pigment showed antiproliferative and proapoptotic effect compared to control. These results suggest that treatment of lymphoma with this pigment might be useful either combine or alone therapy in future. Although Lymphomas are generally not considered as endocrine associated cancers but our group previously demonstrated a gender differences in tumor growth by grafting mice with murine T lymphoma cells. We found that male mice developed larger tumors compared to female mice, a difference that was abolished following ovariectomy, suggesting estrogen regulated growth in vivo. In addition, we looked into the effects of 17 β -estradiol, selective ER α and selective ER β agonists on lymphoma growth in culture and in vivo. Treatment with 17 β -estradiol had minor effects on lymphoma growth, whereas the selective ER β agonists diarylpropionitrile (DPN) and KB9520 showed potent antiproliferative and proapoptotic effect. This highlights a significant role for fungal red pigments and estrogens, particularly ER β signaling, in the pathology of NHL. We also suggest that selective ER β agonists and Fungal pigments might be a new and useful therapeutic approach for treatment of lymphomas.

O.1.3. AMELIORATIVE POTENTIAL OF NITRIC OXIDE DONOR RECONDITIONED STEM CELLS AND CHITOSAN BASED HYDROGEL FOR WOUND HEALING

Muhammad Tariq^{1*}, Zeeshan Akram¹, Afshan Afreen¹

¹Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250, (AJK), Pakistan

Corresponding author Email: tariq.awan@must.edu.pk

Impaired wound healing is a serious problem for diabetic community worldwide and is associated with high incidence of bacterial infection and chronic nerve and blood vessel damage leading to repeated amputation of limbs and organs. Stem cells are extensively used to treat different diseases, such as wound healing and repairing of damage tissues due to their potential to differentiate into different lineage and this therapeutic potential of stem cells can be enhanced by pre-conditioning. Nitric oxide (NO) donor agents such as nitroglycerine have shown great therapeutic potential. Chitosan is a natural, bio-degradable, biocompatible and nontoxic polymer. Chitosan-based hydrogel also acts as a good wound healing and repairing agent and has adsorption properties. It is hypothesized that combination stem cells preconditioned with NO donor agent and chitosan based hydrogel would enhance cutaneous wound healing. Therefore, in the present study bone marrow derived mesenchymal stem cells (BM-MSCs) preconditioned with nitroglycerine along with chitosan hydrogel were used to enhance wound healing process in rabbits. In first step of study wounds were inflicted in rabbits. In second step, BMCs and nitroglycerine pre-conditioned BM-MSCs were used to treat wounds. To assess the therapeutic activity of different treatments, wound contraction, re-epithelization and histological studies were carried out. The results showed that nitroglycerine pre-conditioning BMCs combined with chitosan hydrogel possess significant wound healing potential.

**O.1.4. IN VITRO AND IN VIVO ANALYSIS OF BIOACTIVE COMPOUNDS
ISOLATED FROM *BISTORTA AMPLEXICAULIS***

Andleeb Manzoor¹ and M Sheeraz Ahmad¹

University Institute of Biochemistry and Biotechnology,

Pir Mehar Ali Shah Arid Agriculture University, Rawalpindi Pakistan

Corresponding Author Email: andleebmanzoor1992@gmail.com

Medicinal plants are well known for their bioactive phytochemicals. *Bistorta amplexicaulis* member of genus *Polygonum* comprises many phytochemicals like alkaloids, phenolic compounds. *B. amplexicaulis* a rich source of antioxidant and possess a robust ability to scavenge free radicals. In the present study, an attempt has been made to evaluate ethyl acetate fractions of *B. amplexicaulis* for *in vitro* cytotoxic activity on Hep2c and RD cell lines by MTT assay. The antioxidant effect of ethyl acetate fractions and crude methanolic extract (CME) of *B. amplexicaulis* evaluated for *in vivo* analysis in normal and streptozotocin (STZ) induced diabetic mice. Blab/c mice were divided in different groups as non-diabetic, diabetic untreated and diabetic treated with EA fractions, CME and standard drug Metformin. Diabetes was induced in mice by intraperitoneal administration of STZ (100 mg/kg body weight) for five days. Oral administration of ethyl acetate fractions and crude methanolic extract of *B. amplexicaulis* at a concentration of 200 mg/kg to normal and STZ induced diabetic mice was given for 20 days. The ethyl acetate fractions show cytotoxicity against cancer cell lines. The fractions and crude methanolic extract of *B. amplexicaulis* significantly decreased the levels of blood glucose in diabetic mice. The treatment resulted in a significant increase in superoxide dismutase, catalase, and peroxidase in serum of diabetic mice as compare to diabetic control. These results shows *B. amplexicaulis* possess anticancer and antioxidant properties. Histopathological studies demonstrated protective effect of *B. amplexicaulis* in diabetes by decreasing oxidative stress.

Keywords: *Bistorta amplexicaulis*, antioxidant, anticancer, streptozotocin-induced diabetes

O.1.5. CHRONIC TOXICITY AND BIOCHEMICAL EVALUATION OF METHANOL EXTRACT OF *IPOMOEA HEDERACEA* SEEDS ON RABBITS

Mehjabeen^{1*}, Noor Jahan² and Asma Wazir³

^{1*} Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University for Science, Arts & Technology, Karachi, Pakistan. *E-mail: mehjb1@gmail.com

²Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, 74200, Pakistan.

³ Department of Pharmacognosy, Faculty of Pharmacy Dewan University, Karachi, Pakistan

Corresponding author Email:: mehjb1@gmail.com

Plants belongs to genus *Ipomoea* have traditional medicinal importance. One of specie *Ipomoea. hederacea* locally known as kaladana is used in various ailments and skin preparations. Therefore, Present study is design to evaluate the chronic toxicity of methanol extract of *I. hederacea* seeds. The methanol extract of *I. hederacea* seeds (1mg/kg dose) was administered orally in different groups of animals (rabbits) for 90 days. The extract was evaluated for blood chemistry, liver, cardiac and kidney functions. Tissue histology was performed on liver, heart, kidney and stomach. Antioxidant activity was performed by DPPH assay at different concentrations. These parameters were statistically calculated at $p < 0.05$. The results from hematological and biochemical study revealed that Oral administration of 1mg/kg dose of the extract for 90 days significantly increased the platelet count at ($p < 0.05$). Triglycerides, total cholesterol, low density lipoprotein and very low density lipoprotein were reduced whereas HDL was elevated ($p < 0.05$). The extract also showed slightly reduced hemoglobin level. Creatinine was reduced where as CPK was Increased in treated groups. LDH and CK-MB decreased slightly in male test group. No lethality was observed in any control and treated groups. These results were correlated with tissue histology. Present findings suggested that *I. hederacea* seeds have antioxidant, platelet enhancing, and hypoglycemic effect but for medicinal usage dose and duration need to be monitored to avoid any side effects.

Key words: *Ipomoea hederacae* seeds, biochemical parameters, chronic toxicity, antioxidant

O.1.6. RELATION OF SERUM VITAMIN D WITH INSULIN RESISTANCE IN OBESE FEMALE PRESENTING WITH NORMAL FASTING GLUCOSE LEVELS

Shan Elahi*¹, Sadaf Aslam², Ayesha Ammar², Nayyab Batool Rizvi² and Affia Tasneem¹

¹Centre for Nuclear Medicine (CENUM), Mayo Hospital, Lahore

²Institute of Chemistry, New Campus, University of the Punjab, Lahore

Corresponding Author Email: shan.elahi@gmail.com

Vitamin D maintains glucose homeostasis, enhance beta-cell functioning and improves insulin sensitivity. Obese subjects are at increased risk of both vitamin D deficiency and development of diabetes mellitus (DM). We determined mutual relation of both these factors in local obese female. At CENUM, Mayo Hospital Lahore during February to May 2018, obese female (BMI ≥ 30 Kg/m²) were recruited and necessary information and a 5 ml venous blood was collected from each participant. Weight and height was noted and BMI was calculated. Exclusion criteria were the presence of DM or other systemic disease, taking of anti-hyperglycemic drug, taking of Vitamin D supplementation and pregnancy. Fasting glucose, fasting insulin and 25-OH vitamin D (Vit D) were determined by using commercial kits. Normal ranges for Vit D, fasting glucose and insulin levels were: 20-50 ng/ml, < 110 mg/dl and <14.5 μ IU/L respectively. Insulin resistance (IR) was determined by formula: HOMA-IR = fasting glucose $\times$ fasting insulin/405 (Mattews 1985). A total of 141 obese female (age range: 18-65 year.) were selected for this study. About one-fourth (n = 23; 24.1%) of them had central obesity. Most of them (n=118; 83.7%) were vitamin D deficient (VDD) and only 23 subjects were vitamin D sufficient (VDS). Both VDD and VDS groups were comparable with respect to average age and BMI (P>0.05). In VDS both fasting glucose and insulin and in VDD subjects only insulin was negatively correlated with vitamin D level. The glycemic parameters in VDD and VDS obese subjects were comparable but the percentage of female with HOMA - IR ≥ 2.5 was significantly lower in VDS subjects (21.7% versus 35.6%; p<0.05). Vitamin D sufficiency adequately protects from development of insulin resistance and hence DM in obese female.

Key Words: Vitamin D, Diabetes Mellitus, Obesity, Fasting Glucose, Insulin Resistance

**O.1.7. ANTIMICROBIAL AND BIOLOGICAL SCREENING OF SECONDARY
METABOLITES IN *CITRULLUS COLOCYNTHIS***

Modasrah Mazhar, Muhammad Afzal, Iqra Baig and Shafia Arshad

Faculty of Life Sciences, University of Central Punjab, Lahore

Department of Eastern Medicine and Surgery, Qarshi University, Lahore

Corresponding author Email: modasrah mazhar381@yahoo.com

All the way through the world medicinal plants are considered as the spine for system of medication after the discovery of antibiotic resistance. Medicinal plants act as the conventional source for treating different diseases. Current study was designed for determining the ethnobotanical properties of a well-recognized plant in the traditional medicine i.e. *Citrullus colocynthis*. It was found that extracts of *Citrullus colocynthis* (containing secondary metabolites) are significantly antioxidant with antimicrobial activity against bacterial and fungal cultures. Furthermore to stamp this result HPLC analysis was conducted. Findings of this research are supporting the use of *Citrullus colocynthis* for therapeutic applications as antioxidants and antimicrobial hence techniques applied in this research will be used to explore other bioactive compounds in different medicinal plants.

Key words: Antioxidants, antimicrobial, *Citrullus colocynthis*, HPLC

**O.1.8. ANTIMICROBIAL VALUE OF OIL EXTRACTED FROM SAARA
HARDWICKII AND PELICANUS CRISPUS**

*Maryam Arshad, *Tahira Ruby, **Mirza Imran Shahzad, ***Muhammad Rafay,

****Muhammad Abdullah and *****Aleem Ahmed Khan

* Department of Life Sciences, **University College of Veterinary and Animal Sciences,
University College of Agriculture and Environmental Sciences, *Cholistan Institute of
Desert Studies, The Islamia University of Bahawalpur, Bahawalpur

***** Department of Zoology, Ghazi University, Dera Ghazi Khan

Corresponding author Email: tahira.ruby@iub.edu.pk

Various products obtained from animals may be used for preparation of many traditional medicines and have probably been used since prehistoric times. These products represent as an important alternative for the treatment of many bacterial and viral ailments in many parts of the world, especially in underdeveloped regions. Among Reptiles, *Saara hardwickii* and among birds *Pelicanus crispus* are frequently used for medicinal purpose in Pakistan and India. Their body fats have many therapeutic properties in local folk medicines. Present research work represents antibacterial and antiviral value of body fat of *Saara hardwickii* as well as the ecological implications of its use in traditional medicine. Oil was extracted from body fats located in the ventral region of both these animal using hydrocarbons e.g. n-hexane, methanol, butanol and ethyl acetate as a solvent. The antibacterial activity of lizard oil was tested against standard as well as multi-resistant lines of *Escherichia coli*, *Salmonella aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* either alone or in combination with antibiotics. For antibacterial potential, Ethyl acetate solvent extract showed best zone of inhibition (7mm) with *P. aeruginosa* while Butanol solvent extract showed best zone of inhibition (7mm) with *S. aureus*. For antiviral potential, Butanol and Methanol extract showed best HA (hemagglutination) titer of 04 with NDV and IBV viral strain respectively. The antibacterial activity of pelican oil was also tested against standard as well as multi-resistant lines of *Escherichia coli*, *Salmonella aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* either alone or in combination with antibiotics. For antibacterial potential, Butanol and Methanol solvent extract showed best zone of inhibition (5mm) with *P. vulgaris* and *E.coli* respectively. For antiviral potential, Ethyl acetate extract showed best HA (hemagglutination) titer of 02 with NDV viral strain while n-hexane with IBV viral strain at HA titer of 02.

O.1.9. ROSACEAE FRUITS - A HEALTHY FOOD

Muhammad Afzal, Modasrah Mazhar, Naveed Ahmed

Faculty of Life Sciences, University of Central Punjab, Lahore- Pakistan

Department of Eastern Medicine and Surgery, Qarshi University, Lahore-Pakistan

The *Rosaceae* or *Rose* family is the 19th largest family of flowering plants. The family *Rosaceae* includes many economically and medicinally important fruits such as apple, plum, apricot, peach and pear. *Rosaceae* fruits are rich in bioactive phenolic compounds including phenolic acids, flavonoids, coumarins, vanillic acid, *P*-Coumaric acid, ferulic acid, syringic acid and tannins. These combined phytochemicals present in fruits perform variety of physiological functions and biological activities such as antioxidant, anti-inflammatory, anti-tumor and anti-carcinogenic. This family has a prime importance due to good profile of essential minerals along with amino acids, free sugars, vitamins and organic acids that play an important role in maintaining their quality and determining nutritive value. The identification of compounds was completed using various identification techniques like HPLC, FTIR, GC-MS, LC-MS and NMR. It is observed in this study that low molecular weight organic acids are a useful indicator for quality assessment and authenticity of fruits and fruit-derived products. The present study indicates that intake of phenolic compounds and enzymatic antioxidants through our diet in the form of fruits and vegetables are strongly linked with protection against cardiovascular heart diseases, certain cancer and chronic health disorders.

Keywords: *Rosaceae* fruits, antioxidants, healthy food, phenolic compounds, anti-carcinogenic.

1.10. EXPLORING MEDICAL APPLICATIONS OF NANOSIZED SILVER PARTICLES IMPREGENATED IN FUNGAL EXTRACT AND THEIR CHARACTERIZATION

Saima Faryad and Dr. Bushra Arshad

Department of Biotechnology, Mohi- Ud-Din Islamic University, Nerian Sharif, AJ&K

Corresponding author Email: bushraarshad1988@gmail.com

Nanobiotechnology refers to the ways that are used by nanotechnology to create devices to study biological systems. Nanotechnology is the creation, manipulation and use of materials at the nano size scale (1 to 100 nm). Nanoparticles are considered as building blocks of Nanotechnology. The synthesis of numerous metal nanoparticles is now the most attractive area of research in nanotechnology. Silver has been used since ancient time due to its potent antimicrobial effect and now it is recognized as a non toxic and safe for human beings. Silver nanoparticles (AgNPs) are widely used in several industries because of their unique physico-chemical and antimicrobial properties. For the synthesis of metal nanoparticles there are many chemical as well as physical methods, however, green synthesis or biosynthesis which involve different biological agents is the most emerging and ideal method of synthesis due to several reasons. In present study the synthesis of silver nanoparticles (AgNPs) were reported by using *Aspergillus fumigatus*. These silver nanoparticles were characterized by means of UV-vis spectroscopy, scanning electron microscope (SEM), X ray diffraction (XRD), these measurements confirmed the bio-synthesis of silver nano particles. Results indicate the synthesis of silver nanoparticles in the reaction mixture. Antibacterial activity of silver nano particles were analyzed by using agar well diffusion method and optical density measurement in liquid media. The silver nanoparticles were found to possess significant antibacterial activity against standard test pathogenic gram positive strain, *Staphylococcus aureus* and gram negative strains namely *Shigella dysenteriae* and *Salmonella typhi*.

**O.1.11. BIOACTIVE ANALYSIS AND SEARCH OF NOVEL ANTIMICROBIAL
FAMILY SPECIFIC GENES FROM MEDICINAL PLANTS**

Um-e-Habiba, Raheela Jabeen and Atia Iqbal

The Women University Multan, Pakistan

Corresponding author Email: draheela.9054@wum.edu.pk

The defence mechanism of plants provoked by biotic and abiotic hassles cause the instigation of defensin genes. These defensin genes are key gear for the fabrication of transgenic self-defensive pants and potential natural antibiotics. This rummage of natural weapons, beginning in 90s, is still on the way. This research work also deliberated to chase potential novel defensin genes relying on the antimicrobial potential of the medicinal plants including *Cassia fistula*, *Cymbopogon citratus*, *Albizzia lebecck* and *Saccharum officinarum*. After isolation of quality genomic DNA, PCR was done using defensin primers, which were designed by primer3 using open reading frame of already acknowledged defensin genes of palnts. Defensin genes of *Cassia fistula* and, *Albizzia lebecck* were efficaciously amplified, with size of 150 bp and 300 bp respectively. Both genes were isolated from gel and purified using Thermoscientific genejet gel extraction Kit. After that, genes were sent for sequencing. The sequence results will be prophesied for Insilco analysis to evaluate the possible potential of the purified genes.

**O.1.12. CLINICO -PATHOLOGICAL CHANGES ASSOCIATED WITH INDUCED
NEWCASTLE DISEASE IN NEEM (*AZADIRACHTA INDICA*) SUPPLEMENTED
BROLIER CHICKS**

Muhammad Umar, Farzana Rizvi, Shah Nawaz*, Ayesha Ramzan

and Muhammad Shahzad Shafiq

Department of Pathology, Faculty of Veterinary Science,

University of Agriculture, Faisalabad

Corresponding author Email: malikshahnawaz786@gmail.com

Aim of study was to investigate the effects of Neem (*Azadirachta indica*) supplementation on Newcastle disease (ND) in broiler chicken. During this trail a flock of total 175, one-day old chicks were randomly divided into 5 groups; having 35 broiler chicks in each group. Birds of one group kept as control and no treatment was given. Chicks of other groups were given neem leaves at the prescribed dose @ 8g/kg, in feed from day 3 to 42 of trail. Chicks of rest of groups were challenged with field isolated NDV strain on 30th day of study by sub cutaneous route. All birds were observed twice daily for clinical signs and behavioral changes. At a week interval, seven birds were slaughtered from each group upto 42 day of the trail. Blood from wing vein was collected with and without anticoagulant for hematology and for serum biochemistry. At the end of trail, significant increase in body weight was found in all neem fed groups as compared to control group. There was significant increase in total erythrocyte count (TEC), packed cell volume (PC V), hemoglobin (Hb), serum proteins and globulins level in all neem supplemented groups as compared to control group. There was non-significant difference in total leukocyte count (TLC), serum ALT, serum urea and creatinine of broiler chicks of all neem supplemented groups as compared to control group. From this study it could be concluded that the use of neem has beneficial effects on performance and body weight gain of broiler chickens. Similarly, it has no adverse effects on the internal physiology of broilers and haematological & serum biochemical parameters.

O.1.13. ENDOGENOUS ANTIOXIDANT AND CHEMICAL ASSESSMENT OF POLYHERBAL FORMULATION (PHF) AGAINST VARIOUS HEPATOTOXIC AGENTS IN EXPERIMENTAL ANIMALS

¹Abid Ali and ²Khalil ur Rahman

¹Department of Allied Health Sciences, The University of Lahore, Gujrat Campus

²Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: abid.ali@ahs.uol.edu.pk

Silymarin isolated from the *Silybum marianum* is used clinically for the management of hepatic ailments worldwide and its hepatoprotective potential is also proved in previous study by our research group. Advancement of liver injury characterized by increased levels of enzymes in blood stream and its complication includes bile leak, *hepatic* and perihepatic abscesses, ascites, cirrhosis and decompensated cirrhosis. In order to manage the multiple symptoms of liver injury and reduction in the progression of liver related complication there is need to formulate a herbal combination of medicinal plants to manage the hepatic ailments. In addition to silymarin another plant that has similar therapeutic character is *Rheum emodi* and *Artemisia absinthium* are combined to see the synergistic hepatoprotective effect. The extract of poly herbal combination was standardized using LCMS/MS) to evaluate their hepatoprotective effect on various hepatotoxicant induced in experimental animals. The administration of hepatotoxin, significantly increased the levels of SGPT, SGOT and ALP whereas the levels of protein and albumin were decreased. Treatment with herbal combination showed a strong hepatoprotective activity based on assessing the levels of above described markers. The levels of antioxidants enzymes i.e. SOD, POD and Catalase in medicinal plants treated groups remained in normal limit as in the control group. Histopathological examination of liver tissues of control and treated animals showed significant difference indicating that the herbal combinations successfully restored the liver functions.

O.1.14. OMEGA-3 ENRICHMENT OF POULTRY EGGS THROUGH USE OF FLAXSEED

Asghar Subhani¹, Shaukat Ali Bhatti^{1*}, Gulraiz Ahmad², Tahir Mahmood¹, Shahzad Ashraf¹,
Najam Us Sahar¹, Muhammad Khalid Qadeer¹

¹Institute of Animal and Dairy Sciences, University of Agriculture Faisalabad, Pakistan

² SB Feeds (Pvt.) Limited, Islamabad, Pakistan.

Corresponding author Email: sabhatti60@gmail.com

Saturated and trans fatty acids have negative effect on the human health, however, polyunsaturated fatty acids (PUFAs) have positive effect to prevent from cancer and cardiovascular diseases. Omega-3 and Omega-6 PUFAs compete for metabolism and have antagonistic effects on human health. Hens have a unique ability to accumulate dietary lipid into their egg yolk. Adding flaxseed to layer diets significantly increased yolk omega-3 fatty acid. The study was planned to investigate the effect of flaxseed (FS) on omega-3 fatty acid composition of eggs in laying hens. For this purpose, one hundred and ninety-two (n=192) Lohmann Selected Leghorn (LSL) Lite laying hens at age of 31 week were divided randomly into three groups and fed diets containing three levels (0, 5 and 10%) for 10 weeks with first two week as adaptation period. Eggs were collected, counted and weighed daily. Egg fatty acids were analyzed by gas chromatography technique. Data were analyzed using mixed procedure of SAS. The effect of bird was taken as random. Treatment and weeks were taken as dependable variables. Means were compared using Tukey's Test. Alpha-linolenic acid contents in eggs increased with increasing level of FS while linoleic acid (omega-6), decosahexanoic acid (DHA) and eicosapentanoic acid (EPA) contents were not affected by dietary treatments. It was concluded that supplementation of FS in layer diet significantly enhanced the linolenic acid contents.

Keywords: flax seed, omega-3 fatty acids, eggs, layer birds

O.1.15. EFFECT OF ALOE VERA POWDER AGAINST ACNE VULGARIS

Aysha Sameen, Maria Manzoor, Muhammad Issa Khan and Amna Sahar

National Institute of Food Science and Technology, University of Agriculture, Faisalabad

Corresponding author email: ayshasameen@uaf.edu.pk

Acne vulgaris is common skin disease characterized with chronic inflammatory and multifactorial pathogenesis. All age groups are affected mainly teenagers and adults once in their lives. For treatment, a number of standard oral and topical therapies are used but these drugs induce serious complications. *Aloe vera* herb is used in different cultures and pharmaceutical industries for its therapeutic agents. It contains several active components and antimicrobial properties. To keep in view the unique role of *Aloe vera* powder, study was designed to evaluate the effect of *Aloe vera* in group of patients with mild to severe acne vulgaris. Powder was prepared from *Aloe vera* and filled in capsules. Human subjects were recruited and divided in 3 groups. Subjects from study 1 and 2 consumed a capsule each day. Subjects were clinically examined at DHQ hospital Faisalabad. In control group T₀ who didn't receive any medication the initial mean value of acne vulgaris severity was 2.3 ± 0.8 and after 15 days was 2.1 ± 0.5 while the final value was 2.2 ± 0.6 . In experimental study T₁ with 15mg capsule the initial mean value was 2.9 ± 0.8 and after 15 days of study 2.6 ± 1.2 , while the final value was 2 ± 0.8 . Whereas in experimental group T₂ who received 30mg capsule the initial mean value was 2.9 ± 0.8 and after 15 days was 2.3 ± 1.1 . Mean value after 30 days significantly reduced to 0.9 ± 0.7 . It proves that *Aloe vera* has therapeutic effect against acne vulgaris.

Keywords: Acne, Aloe vera, skin disease

**O.1.16. STUDY OF THE ANDROGENIC POTENTIAL OF ANACYCLUS
PYRETHRUM ROOTS EXTRACT IN ADULT MALE RATS**

Muhammad Riaz¹, Fatima Yousaf², Muhammad Shahid², Asma Irshad³, Ghulam Rasool¹

¹Department of Allied Health Sciences, Sargodha Medical College, University of Sargodha,
Sargodha-Pakistan.

²Department of Biochemistry, University of Agriculture, Faisalabad-Pakistan

³Centre of Excellence in Molecular Biology, University of the Punjab, Lahore-Pakistan

Corresponding author Email: riazmlt786@gmail.com

Anacyclus pyrethrum belongs to Asteraceae plant family which is found in Asian countries. This plant is being used in traditional system of medicines for variety of diseases including infertility. The current study was conducted to explore the androgenic potential of methanolic roots extract of Anacyclus pyrethrum by determining the testosterone concentration in serum and the testicular histology of male rats. The methanolic roots extract was evaluated for various biological activities i.e. antioxidant potential and cytotoxic activities. Animals were divided into six study groups of four animals each including normal control (A), toxic group (B), positive control (Testosterone treated) (C) and test groups (D, E and F). Test groups D, E and F received 50, 100 and 200 mg/Kg bw A. pyrethrum roots extract for six weeks. Body, testis and relative testis weight of rats were recorded. Testosterone level was determined through ELISA kit method. Testicular histology was performed by H & E staining. Significant ($p < 0.05$) variation in body and relative testis weight were observed in A. pyrethrum roots extract treated animals compared to control rats. Significant ($p < 0.05$) increase in testosterone concentration was found in extract treated rats. Histological study revealed that A. pyrethrum roots restored the histoarchitecture of rat testis in CCl₄ intoxicated rats. The study concluded that A. pyrethrum roots have testosterone like activity probably due to the presence of bioactive phytochemicals and might enhance male fertility.

O.1.17. LEVEL OF TRACE ELEMENTS AND STEROID HORMONE IN POLYCYSTIC OVARY SYNDROME

*Muhammad Jahangeer¹, Zahed Mahmood¹, Maliha Batool¹, Sarmad Ahmad Qamar²,
and Ghazia Fatima¹

[1] Department of Biochemistry, Government College University Faisalabad, Pakistan.

[2] Department of Biochemistry, University of Agriculture Faisalabad, Faisalabad, Pakistan.

*Corresponding Author: Muhammad Jahangeer (Research Associate) GCUF

Corresponding author Email: rajahangeer@gcuf.edu.pk

Polycystic ovary syndrome is an endocrine dysfunction and abnormal androgen production. There is almost 6.5–8% females affected at the age of puberty. Approximately 4 to 12% females are affected by this syndrome at their reproductive age. Symptoms of PCOS include obesity, pelvic pain, menstrual irregularities, acne, hirsutism, and alopecia. In polycystic ovary syndrome there is also an important role of the oxidative stress. Various studies investigate the relation of oxidative stress to polycystic ovary syndrome. Symptoms of polycystic ovary syndrome include obesity, acne, hirsutism, infertility and alopecia etc. we aimed to find out level of hormones, level of heavy metals and check biochemical profile. Fifteen patients and five healthy females under the age of 20-40 years selected. So, by the investigation it is concluded that the level of testosterone, FSH, LH and insulin was significantly higher in patients than the healthy females and because of this the patients suffer from menstrual irregularities, obesity, infertility and hirsutism. Level of different elements also checked out. Level of zinc and copper was significantly higher in the patients while level of magnesium, cobalt and cadmium differ a little bit. Biochemical parameters also checked during the research. Glucose and triglycerides level also have significant increase in disease condition and it resultantly increase level of cholesterol and which cause obesity. Mean and standard deviation of all parameters was determined which show the level difference in polycystic ovary syndrome (PCOS) and control group.

Keywords: Polycystic Ovary Syndrome, Trace Elements, Steroid Hormones, Infertility, obesity.

O.1.18. INVESTIGATION OF *FELINE PANLEUKOPENIA VIRUS* ANTIBODIES IN DIARRHEIC CAPTIVE FELINES IN DISTRICT FAISALABAD, PAKISTAN

Farrah Deeba^{1*}, Anas Sarwar Qureshi², Muhammad Haleem Tayyab¹, Naureen Rana³,
Ayesha Anwar³, Khurram Ashfaq¹

¹Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad

²Department of Anatomy, University of Agriculture, Faisalabad

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

Corresponding author Email: farrah.deeba@uaf.edu.pk

Feline infectious enteritis due to Feline panleukopenia virus (FPV) is a highly fatal disease of domesticated and wild felines with worldwide distribution. The present study was devised to establish prevalence of antibodies for feline panleukopenia virus in different breeds of domesticated cats in district Faisalabad. In 2016-17, blood samples were drawn from 50 cats with history of enteritis from all areas of district Faisalabad for detection of antibodies to FPV infection. All the sera harvested by centrifugation were subjected to hemagglutination inhibition assay (HIA). Out of 50 samples tested, 37 samples were found HIA positive for FPV infection with overall 74% prevalence in cats in district Faisalabad. Multivariable analysis revealed significant association ($p < 0.05$) of seroprevalence of FPV with juvenile age group (<1year), lack of vaccination and enteritis history, while breeds and sex were considered as non-significant factors with $p > 0.05$. This study concluded higher prevalence of FPV antibodies in domestic cats of district Faisalabad and suggested proper vaccination protocol to avoid fatal outbreaks in household cats.

Keywords: Panleukopenia, Hemagglutination assay, Enteritis, Felines, Seroprevalence, Faisalabad

O.1.19. ASSESSMENT OF MEDICINAL IMPACT OF *CALOTROPIS PROCERA* SUPPLEMENTED DIET THROUGH HAEMATOLOGY, GUT MICROBIAL DIVERSITY AND IMMUNE RESPONSE IN BROILER CHICKS

Syeda Sadaf Zahra¹, Nargis Naz¹, Gulnaz Afzal², Ghulam Mustafa³ and Shumaila Kiran⁴

¹Department of Botany, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

²Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

³Department of Biochemistry, Government College University, Faisalabad, Pakistan

⁴Department of Applied Chemistry, Government College University, Faisalabad, Pakistan

Corresponding author Email: gulnaz.afzal@iub.edu.pk

The effects of *Calotropis procera* Linn. leaves supplemented diet in poultry chicks on haematology, gut microbial diversity and immune response were evaluated in this study. Ross 308 broiler chicks (a total of 400 birds) were randomly assigned five dietary treatments with four replicates in a completely randomized designed with 20 pens containing 20 chicks in each pen. The chicks were fed diet containing 0, 12.5, 25, 37.5 g/half bag dried *Calotropis* leaves along with Enramycin 3.125, 2, 1, 0 g/half bag and designated as T1, T2, T3, T4 and T5. Supplemented diet along with fresh clean drinking water was supplied ad libitum throughout the experimental trial of 35 days. On the last day of trial 2 birds per pen were selected for blood samples for hematology and gut microbial diversity evaluation. The results were found significant ($P<0.05$) for hemoglobin (Hb), total leucocytes (TLC), total red blood cells (TRBCs), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) in hematology and significant ($P<0.05$) for total coliform (TC), clostridium (Clos) and Bifido in gut microbial diversity. However, the plant material did not show significant results ($P<0.05$) for immunological response against New Castle and Infectious Bronchitis diseases in chickens. It was concluded that the *C. procera* has no negative effects on poultry haematology and gut microbial diversity but it improves chick's health.

Keywords: *Calotropis Procera*; broiler chicks; hematology; microbial diversity; New Castle; Infectious Bronchitis

O.1.20. EVALUATION OF NITRIFICATION INHIBITION POTENTIAL OF THIOUREA, 1,2,4-TRIAZOLE AND ACETOHYDROXAMIC ACID IN WHEAT AND RICE CROP

^aMuhammad Asif, ^aNazish Jahan, ^bKhalil-Ur-Rehman, ^aZill-e-Huma and ^aZeeshan Khadim

^aDepartment of Chemistry, University of Agriculture, Faisalabad, Pakistan

^bDepartment of Chemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: east2165@gmail.com

A major portion of urea applied for enhancing crops yield and quality is losing every year in the form of nitrates leaching, N₂O and NH₃. The present study was conducted for the evaluation of nitrification inhibition potential of three nitrification inhibitors, acetohydroxamic acid, 1, 2,4-triazole and thiourea in wheat and rice crop. In the first phase, physio-chemical properties were determined and in second phase, the nitrification inhibition potential of three inhibitors was assessed. The inhibition potential was evaluated in the rice and the wheat growing seasons of 2017-18. The three treatments of each inhibitors were applied having conc. of T₁: 0.1%, T₂: 0.25% and T₃: 0.5% (w/w). The urea was applied as control at a rate of 0.782gN/6kg soil (120 kg N/ha) by using (RCBD) with three replications of each treatment. Methods like, Kjeldhal, Spectrophotometry and Flame photometry were used. The average values of different physio chemical parameters were obtained after analysis of 15 soil samples selected from the Faisalabad District were as follows; pH (8.42±0.08), EC (476±0.02mS/cm), N (48.2±0.001mg/ 1g of soil), P (121.8±0.003mg/ 5g soil), soil moisture contents (0.382±0.012g/ 10g soil), K (103±0.024mg/ 10g soil) and soil organic matter (2.48±0.01%). The results obtained for nitrates estimation showed that in rice crop the T₃: 0.5% and T₂: 0.25% treatments of thiourea showed 41.65±2.78mg and 95.75±1.35mg NO₃⁻-N losses in the 1st leachate. Similarly, both these treatments of thiourea also showed 0.626±0.001mg and 1.481±0.0013mg nitrate leaching losses in the 2nd leachate respectively. The T₃: 0.5% treatment of acetohydroxamic acid showed 72.85±1.01mg and 0.784±0.002mg of leaching losses in the 1st and 2nd leachates obtained from rice crop. For wheat crop, T₃: 0.5% of 1,2,4-triazole, T₂: 0.25% of thiourea and T₂: 0.25% treatment of acetohydroxamic acid was showed best nitrification inhibition potential. It was concluded that all the nitrification inhibitors reduced nitrogen losses in the form nitrates into the ground water and the physio chemical properties have also played an important role in nitrogen losses from soil through various mechanisms.

Key words: urea, nitrates, nitrification inhibitors, wheat and rice crop

**O.1.21. ANTIMICROBIAL AND ANTIFUNGAL PROPERTIES OF SKIN MUCUS
FROM FRESH WATER CULTIVABLE *CYPRINUS CARPIO*.**

Zaheer Ahmed¹, Muhammad Anjum Zia¹, Ghulam Akbar¹, Yasmeen Batool¹, Hadia Naseem¹
Mehwish Kanwal¹, Abid Ali¹

¹Enzyme Biotechnology Laboratory (EBL), Department of Biochemistry,
University of Agriculture, Faisalabad, Punjab 38040, Pakistan.

Corresponding author Email: mbczaheer@hotmail.com

Fish face heavy pathogenic load in their natural habitat. Epidermal mucus protect the fish from pathogenic effect. The present study was conducted to determine antifungal and antimicrobial potential of epidermal mucus of *Cyprinus carpio*. For this purpose, mucus collected from ten wild habitats (River Ravi) and ten control sites (pond reservoir). *Cyprinus carpio* were tested against pathogenic bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus equisimilis*, *Pseudomonas putida*) and pathogenic fungal (*Aspergillus terreus*, *Aspergillus niger*, *Trimates versicolor*) strains by disc diffusion method. The mucus secreted by the epidermis of wild site of *Cyprinus carpio* exhibited higher antifungal and antibacterial activity than control site of fishes except *Trimates versicolor* that remain unaffected. Ciprofloxacin and ketoconazole were used as positive control for both of the bacterial and fungal strains respectively. The study also determined the protein contents of fish mucus by Bradford assay using bovine serum albumin as standard. The protein content was higher (1.90 mg/mL) in the crude mucus of wild site of fish than the protein contents (1.04 mg/ml) of control site of fish. Protease activity found higher (1.97 Umg⁻¹) in the riverine fish mucus as compared to the mucus of pond fish (1.71 Umg⁻¹) assessed by Casein hydrolysis test. In this investigation, we isolated and detected protease from the skin mucus constituents of *Cyprinus carpio* using a series of ion exchange column chromatography steps then measured molecular mass of protease by SDS-PAGE. The present findings suggest that *Cyprinus carpio* mucus having very good antimicrobial activity against pathogenic microbes.

Keywords: Antimicrobial, *Cyprinus carpio*, Skin mucus, Protease activity.

O.1.22. PREVALENCE OF HYPOTHYROIDISM AND AUTOIMMUNITY IN OBESE WOMEN

Nazish Saleem*¹, Shan Elahi² and Nayyab Batool Rizvi¹

¹Institute of Chemistry, New Campus, University of the Punjab, Lahore

²Centre for Nuclear Medicine (CENUM), Mayo Hospital, Lahore

Corresponding Author: Nazish Saleem Lecturer, Institute of Chemistry, New Campus,
University of the Punjab, Lahore

Corresponding author Email: nazishsaleem34@gmail.com

Objective: Obese subjects particularly female are at increased risk of endocrine disorders. We determined hypothyroidism and thyroid autoimmunity (TAI) in local obese female referred to CENUM for thyroid function evaluation. Patients and Methods: At CENUM, Mayo Hospital Lahore during February to May 2018, obese female (BMI ≥ 30 Kg/m²) were recruited and necessary information and a 5 ml venous blood was collected from each participant. Weight and height was noted and BMI was calculated. Exclusion criteria were the presence of known thyroid disorder or any systemic disease. Serum concentration of FT₄, FT₃ and TSH were estimated by radioimmunoassay technique. Normal ranges of serum FT₄, FT₃ and TSH were 11.0-23.0 pmol/L, 2.5-5.8 pmol/L and 0.3-5.0 mIU/L. Hypothyroidism was considered when TSH was more than upper and FT₄ was lower than lower limit. A serum thyroid-peroxidase antibodies (TPO-Ab) titer ≥ 20.0 IU/ml was considered AIT positive. Results: A total of 265 obese women and as many normal weight women were enrolled. Their mean (+ SD) age and height was comparable ($P > 0.05$) but average weight, BMI, hip circumference, waist circumference and hip-waist ratio was significantly higher in obese women ($P < 0.05$). Hypothyroidism was detected in 30 (11.3%) patients among obese and 19 (7.2%) patients from control group. Overt hypothyroidism was observed in 19 (7.2%) obese and 9 (3.4%) control women. Subclinical hypothyroidism was detected in 11 (4.2%) obese and 9 (3.4%) control women. Thus incidence of hypothyroidism was more in obese female than control group. A comparison of mean thyroid hormone parameters of euthyroid obese and control women showed that mean FT₄ was significantly lower in obese euthyroid women while mean levels of FT₃ and TSH were comparable. Prevalence of TAI was also comparable in obese and normal weight women (13.1% versus 12.7%; $P > 0.05$). Conclusion: Obese women are increased risk for development of hypothyroidism and thyroid autoimmunity.

O.1.23. FORMULATION OF *AMOMUM SUBULATUM* NANOSUSPENSION AND EVALUATION OF ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITION POTENTIAL

Munazza Rashid^a, Nazish Jahan^a and Khalil-ur-Rehman^b

^aDepartment of Chemistry, ^bDepartment of Biochemistry, University of Agriculture
Faisalabad, Pakistan

Nanosuspension plays propitious role to increase therapeutic effects of drugs and overcome the problems related to the delivery of poorly water soluble drugs. It is distinctive because of its benefits commercially and environment friendly. *Amomum subulatum* possess medicinal properties including increase of appetite, acts as antidote against poison, antioxidant, antifungal, stimulant, causing the dermal cells contraction and cardioprotective. It is reported to control high blood pressure by inhibiting the release of angiotensin converting enzyme. Less solubility and erratic bioavailability minimize the therapeutic effect of plant. Nanosizing enhances the dissolution velocity and saturated solubility of drug particles. The main objective of present research was to enhance the angiotensin converting enzyme inhibition potential of *Amomum subulatum* by formulating its nanosuspension. *Amomum subulatum* nanosuspension was prepared by anti-solvent precipitation method. Different stabilizers (HPMC, PEG 400, SLS, PVA, PVP and Tween 80) were screened to choose the best one. Sodium lauryl sulphate was selected for *Amomum subulatum* nanosuspension formulation. The important parameters, concentration of stabilizer and volume of anti-solvent were optimized by using Central Composite Design of Response Surface Methodology. The prepared nanosuspensions were characterized by zeta sizer and scanning electron microscopy (SEM) and evaluated for their zeta potential, particle size, particle size distribution (PDI) and morphology. Nanosuspension with 136nm particle size and 0.215 PDI value was selected for further *in vitro* study. *Amomum subulatum* used as potential source for developing natural ACE inhibitors through hydrolyzing hippuryl-L-histidyl-L-leucine (HHL) assay. ACE enzyme was extracted from rabbit lungs and its activity was evaluated *in vitro*. Antioxidant potential carried out by different assays showed greater percentage antioxidant potential of optimized nanosuspension as compared to the crude extract. Results of antimicrobial activity showed greater zone of inhibition for nanosuspension against *E.coli* and *B.subtilus* strains as compared to crude extract. Scanning electron microscopy showed spherical shaped nanoparticles with smooth topology which confirmed formulation of nanosuspension. The results suggested that nanosuspension enhance the therapeutic efficiency of *Amomum subulatum* significantly ($p>0.05$).

TRACK 2

**PHARMACEUTICAL BIOCHEMISTRY,
DRUG DEVELOPMENT AND
DELIVERY SYSTEMS**

TRACK COMMITTEE

- 1. PROF. DR. TAHIRA IQBAL (CONVENER)**
- 2. DR. IMTIAZ MEHMOOD TAHIR**
- 3. DR. RAZIYA NADEEM**
- 4. DR SABA JAMIL**

KEYNOTE LECTURES

TRACK 2

K-2.1. POLYMER BASED CANCER NANO-THERAPEUTICS

Dr Abida Raza, Principal Scientist

Group Leader, NILOP Nanomedicine Research Laboratories

National Institute of Laser and Optronics

Pakistan Atomic Energy Commission, Islamabad

Cancer is amongst the leading cause of death across the globe. In last few decades, remarkable breakthrough has been achieved in developing the strategies in better understanding of cancer prognosis, diagnosis and treatment. These developments have decreased the cancer mortalities during last 5 years, but still mortality is high. This high mortality is because of inability of the drug carriers to deliver anticancer agent to its specific site and reducing the dose dependent adverse effects. The quest for nanotechnology based Novel Drug Delivery System (NDDS), has paved the way to develop new drug delivery systems with enhanced biodegradability and biocompatibility. The nanocarriers have potential to carry the drug molecule safely and efficiently to the target site thus reducing the side effects, especially in case of drugs having narrow therapeutic index or cytotoxic effects. Polymeric nanoparticles have proven to be of more interest when compared with metallic and lipid based nanoparticles because of number of reasons like ease of fabrication, high drug loading and encapsulation efficiency, encapsulation of hydrophilic, hydrophobic, charged and macromolecules and above all their greater biodegradation and biocompatibility. These engineered nanoparticles have shown tremendous applications in chemotherapy, a widely used technique to treat millions of patients, predisposed by the cellular sensitivity. Current presentation will highlight the use of polymer as anticancer drug delivery system at NILOP Nanomedicine Research Laboratory.

K-2.2. DEVELOPMENT IN NANOPARTICLES RESEARCH FOR BIOLOGICAL APPLICATIONS

Irshad Hussain

Department of Chemistry & Chemical Engineering, SBA School of Science & Engineering (SSE), Lahore University of Management Sciences (LUMS), DHA, Lahore Cantt-54792, Pakistan.

Metal/metal oxide nanoparticles and nanoclusters have been recognized as an important class of materials whose properties can be tuned by controlling their nanoscale features. A control over their size, shape and surface chemistry is, therefore, extremely important to use them for any potential applications in biomedical sciences. In this regard, we have demonstrated several reproducible protocols to prepare metal nanoparticles from 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 using template-based and template-less strategies. The functionalized metal/metal oxide nanoparticles/ nanoclusters possess interesting optical, recognition and catalytic properties and we are now focusing more on their applications in biological sciences especially those in bio-sensing (bacterial detection), bio-imaging, drug delivery, improved bioavailability and targeted delivery of drugs. This talk would be an overview of our interdisciplinary research activities to synthesize metal nanoparticles/nanoclusters with controlled nanoscale features and surface chemistry, possessing unique chemical and physical properties, and their diverse applications in biological sciences.

ORAL PRESENTATIONS

TRACK 2

O.2.1. DEFENSIVE EFFECTS OF NUCIFERIN AND NORCOCLAURINE ON ACETYLCHOLINESTERASE AND ANTIOXIDANT ENZYMES IN ALLOXAN INDUCED DIABETIC ALBINO RATS

Shahnaz Khan,¹ Hidayatullah Khan,^{1*} Farman Ali Khan², Afzal Shah¹, Shaista Parveen¹,
Muhammad Ashfaq khan¹, Inam Ullah Khan¹

¹Department of Chemistry, University of Science and Technology, Bannu (28100) KP,
Pakistan

²Department of Chemistry, Shaheed Benazir Bhutto University, Sheringal, Dir (18000),
KP, Pakistan

The present study investigates the antidiabetic, antiacetylcholinesterase and antioxidant enzymes effects of nuciferin and norcoclaurine from *N. nucifera* seeds in diabetic rats. The alloxan (100 mg/kg b.w) induced diabetic rats (200-250 g) were divided into 7 groups (n = 6). Group I; normal, Group II; diabetic, Group III; standard glabenclamide, Group IV-VII were methanolic crude (100, 200 mg/kg), nuciferin and norcoclaurine (10 mg/kg b.w.), received for 15 days in dose dependent manner. Different tests included glucose, body weights, enzyme assays i.e. superoxide dismutase, catalase, lipid peroxidation, glutathione and acetylcholinesterase were performed. Nuciferin and norcoclaurine significantly reduced blood glucose ($P < 0.05$) and restored body weight in diabetic rats. Diabetes caused decrease in the antioxidant enzymes level, nuciferin and norcoclaurine (10 mg/kg) significantly increased the antioxidant enzymes in diabetic groups. While, significant increase in TBARS level was observed in diabetic group. Nuciferin and norcoclaurine (10 mg/kg) prevented this increase of diabetic animals ($P < 0.05$). AChE levels were significantly decreased in blood and brain of diabetic group ($P < 0.05$). Our results demonstrated that Nuciferin and norcoclaurine improved memory and interfered with the cholinergic signaling. These observations suggesting a promising use of *N. nucifera* in treatment of Alzheimer's disease and oxidative stress.

Keywords: Nuciferin, norcoclaurine, antidiabetic, antiacetylcholinesterase, antioxidant enzymes effects, *N. nucifera* seed

O.2.2. MESOPOROUS NANO-BIOACTIVE GLASS FOR THE BONE TISSUE REGENERATION AND DELIVERY OF IMATINIB ALONG WITH IN VITRO INHIBITORY EFFECTS ON CANCER CELLS

Muhammad Shoaib^{a*}, Aamer Saeed^a, Muhammad Saif Ur Rahman

and Muhammad Moazzam Naseer^a

^ADepartment of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan

^BClinical Research Center, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, People's Republic of China

For treatment of bone cancer, controlled drug delivery is one of the important methods. Bioactive scaffolds are widely used biomaterials due to their usefulness in localized drug delivery. The aim of this study was to develop mesoporous bioglass (MBG) with improved bioactivity and controllable drug delivery rate. By using pluronic 123 (P123) as a template, a facile sol-gel route was employed for the synthesis of MBG nanoparticles (nps). The composition of the prepared sample was estimated by using energy dispersive X-ray spectroscopy (EDX). These nanoparticles demonstrated the specific surface area of 310 m²/g and pore size of 13 nm as measured by brunauer-emmett-teller (BET) and barrett-joyner-halenda (BJH) method, respectively. The spherical shape of nps was confirmed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Imatinib (IMT); an anti-cancer drug was loaded with the efficiency of 77.59%. The drug release kinetics were precisely controlled by changing the ph (4.4 to 10.4) as well as drug loading concentration (0.2-1.0 mg/ml). The maximum cumulative drug release of 81% was observed over a time period of 250 h at ph of 4.4. Importantly, significant inhibitory effects on the viability of the MG-63 osteocarcinoma cancer cells at 12.19 µg/ml of IMT-MBG were observed. Furthermore, MBG demonstrated ionic dissolution with the release of Ca, K, Si, Na, and P ions upon immersion in simulated body fluid (SBF), which support the formation of hydroxycarbonate apatite (HCA), as confirmed by wide-angle X-ray diffraction (WAXD) pattern and fourier transform infrared (FTIR) spectroscopy. These features proved that IMT-MBG system is effective for bone tissue regeneration and bone cancer treatment.

Keywords: Mesoporous; bioglass; anti-cancer; bone cancer; ph-responsive; tissue regeneration; hydroxycarbonate.

O.2.3. ISOLATION, PURIFICATION, STRUCTURAL ELUCIDATION & OF ANTIBACTERIAL SECONDARY METABOLITES FROM MARINE BACTERIA AGAINST MDR STRAINS OF HUMAN ORIGIN

Bushra Uzaira¹, Nyla Jabeen², Barkat Ali Khan³,

^{1 & 2} Department of Bioinformatics and Biotechnology, International Islamic University, Islamabad, Pakistan

³ Faculty of Pharmacy and Alternative Medicine, Gomal University D.I Khan, KPK, Pakistan

The emergence and spread of antimicrobial resistance continues unabated around the world, leaving devastating health and economic outcomes in its wake. Those consequences will multiply if collaborative global action is not taken to address the spread of resistance. To combat this issue new antimicrobials are urgently needed. Fifteen microbial strains were screened for their capacity to produce antimicrobial metabolites of pharmaceutical interest. These strains were associated to the brown seaweed *Pelvetia canaliculata* (Linnaeus) attached to the rocks of Sonmiani Beach (Karachi, Pakistan). bacterial strains were isolated from sea weeds attached to rocks of Baluchistan coast line using marine agar 2216 and screened for antibacterial activity by agar well diffusion method and crude extract was made and antimicrobial metabolites were purified using silica gel column and structure of pure compound was elucidated using spectroscopic techniques. Crude extract filtrates from CMG 2180 strain, grew on ZMA medium, showed the most remarkable antimicrobial activity, and thus was chosen for further examination. The identification of CMG 2180 as a probable new type strain of the *Actinobacterium Kocuria marina* was based on phenotypic aspects and biochemical characteristics (e.g. halotolerant Gram-positive micrococcoid) as well as on the nucleotide sequence analysis of its full-length 16S rRNA gene showing the highest similarity with the type strain KMM 3905 (GenBank accession number EU073966). Interestingly, a unique UV-bioactive compound, for which the name of kocumarin was proposed, was isolated and purified from CMG 2180 strain's crude extracts by flash silica gel column chromatography and TLC/HPTLC. Using routine methods, kocumarin demonstrated prominent and rapid activities against all tested fungi and pathogenic bacteria including MRSA. Its chemical structure was unraveled by 1D and 2D-NMR spectroscopy as 4-[(Z)-2 phenyl ethenyl] benzoic acid. Taken together and to the best of knowledge, our in vitro data report both a possible new *Kocuria* type strain and the breakthrough discovery of kocumarin, a promising antibiotic exerting quick bactericide and permanent spore inhibition effects. Eventually, after in vivo validation, kocumarin could be produced at high-scale from crude extracts to treat newly infected or microbial resistant patients and/or clean environmental surfaces in order to prevent nosocomial infections.

O.2.4. ANTIPLATELET ACTIVITY OF THUJA OCCIDENTALIS CRUDE EXTRACT AND PARACETAMOL

Noor Jahan^{1*}, Mehjabeen²,

^{1*}Department of Pharmacology, Dow College of Pharmacy, Dow University of Health
Sciences, Karachi, 74200, Pakistan

²Department of Pharmacology, Faculty of Pharmacy, Federal Urdu University for Science,
Arts & Technology, Karachi, Pakistan.

Corresponding author Email: noor.jahan@duhs.edu.pk

Thuja occidentalis (Arbor vitae, Yellow cedar, tree of life) belongs to the family Cupressaceae. The leaves of *Thuja* contain volatile oil (with up to 60% thujone), flavonoids, wax, mucilage and tannins. The established medicinal uses are: anti-viral, expectorant, anti-catarrhal, counter-irritant and diuretic. It is prescribed for both internal and external use in the treatment of warts and polyps as well as for treating amenorrhea. It is an intensively used medicinal plant in Pakistan in alternative system of medicine. The present antiplatelet activity was carried in vitro on the sub-threshold doses of paracetamol and *Thuja occidentalis* extract against arachidonic acid induced platelet aggregation using dual channel chronolog lumi aggregometer. In this study *T. occidentalis* and paracetamol extract sub-threshold dilutions were used with slight modification in solvent system while aspirin was used as standard drug. The results exhibited that *T. occidentalis* extract at 1ng/ml potently inhibits AA-induced human platelet aggregation (82%) in comparison to paracetamol (79%) and aspirin (82%). On the basis of our previous and present results it can be concluded that *T. occidentalis* has diversified therapeutic effect and is beneficial in the treatment of various pathophysiological conditions associated with cancers, kidneys, skin, respiratory tract and digestive system.

Keywords: *Thuja occidentalis*, paracetamol, aspirin, antiplatelet

O.2.5. ABSORPTION KINETICS OF FIXED –DOSE COMBINATION OF SITAGLIPTIN AND METFORMIN IN HEALTHY HUMAN

Mateen Abbas*,¹ and Abdul Muqet Khan²

^{1,2}Quality Operations Laboratory (QOL), University of Veterinary and Animal Sciences
(UVAS), Lahore, Pakistan.

Type-2 diabetes mellitus has become a huge socioeconomic global burden over the last few decades and its prevalence is still increasing. The combined use of sitagliptin and metformin proved to be more effective treatment for type 2 diabetes than a single drug treatment. It is a dipeptidyl peptidase-4 inhibitor and biguanide combination, suggested for treatment of type 2 diabetes. The present work was designed to study the absorption kinetics of fixed-dose combination (FDC) of sitagliptin and metformin in healthy human. After preliminary screening 24 young healthy male volunteers (Average age = 22 years) were selected to participate in this study. Each volunteer was orally administrated a single fixed dose tablet of sitagliptin and metformin 50/500 mg. After predefined time intervals blood sample were collected in the Vacuette[®] tubes from each volunteer and centrifuged for separation of plasma. Amount of sitagliptin and metformin were determined using a validated High Performance Liquid Chromatographic (HPLC) method. The plasma concentration versus time data was used to calculate the absorption kinetics using Equivtest PK software. Maximum plasma concentration (C_{max}) of sitagliptin (278.2 ± 45.6 ng/ml) and metformin (1220 ± 58.8 ng/ml) were observed at 3.67 ± 1.41 hours and 2.88 ± 0.62 hours, respectively. Average area under curve (AUC_{0-t}) of sitagliptin and metformin were 3513 ± 1887 $\mu\text{g.h/ml}$ and 8199 ± 413 $\mu\text{g.h/ml}$, respectively. Average half-life of sitagliptin and metformin (50/500 mg FDC) were 20.21 ± 13.1 hours and 7.18 ± 7.66 hours, respectively. The results indicated the well absorption response of sitagliptin/metformin FDC tablets.

Keywords: Sitagliptin, metformin, HPLC, kinetics, absorption

O.2.6. BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES OF FUNGAL STRAIN (PENICILLIUM OXALICUM) AS POTENTIAL ANTIBIOTICS

Nosheen Feroze and Dr. Bushra Arshad

Department of Biotechnology, Mohi- Ud-Din Islamic University, Nerian Sharif, AJ&K

Corresponding author Email: bushraarshad1988@gmail.com

Biotechnology is the use of living things especially cells and bacteria for production of various products for benefiting human beings. Nanobiotechnology is the convergence of engineering and molecular biology that is leading to a new class of multifunctional devices and systems for biological and chemical analysis with better sensitivity and specificity and a higher rate of recognition. At the nanoscale level, the properties of matter are significantly different from their macroscopic bulk properties. Silver is a nontoxic, safe inorganic antibacterial agent and can serve as replacement of antibiotics. Present research was based on biogenic synthesis of silver nanoparticles as potential antibiotics. Biological method was used for the biogenic synthesis of silver nanoparticles from fungal metabolite of penicillium oxalicum. For characterization of these biosynthesized silver nanoparticles, different analytical techniques X-ray diffraction and scanning electron microscopy were used. Further the antibacterial activity of biosynthesized silver nanoparticles was checked against Staphylococcus aureus, Shigella dysentery and Salmonella typhi by using well diffusion method and U-V visible spectrophotometer. Maximum zone of inhibition for Staphylococcus aureus 17.5 Shigella dysentery 17.5 and Salmonella typhi was 18.3mm. The biosynthesized silver nanoparticles of Penicillium oxalicum show excellent antibacterial activity. So it was concluded from our results that biosynthesized silver nanoparticles have significant potential for a wide range of biological applications such as antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammation.

**O.2.7. SYNTHESIS OF NANOSTRUCTURED, CHARACTERIZATION
& IMPROVEMENT OF BIOAVAILABILITY, PHARMACOKINETICS
IN VITRO AND VIVO STUDIES**

Murtaza Hasan PhD

Department of Biochemistry & Biotechnology,

The Islamia University, Bahawalpur, Pakistan

Synthesis of nanomaterials is being gained extensive attention in the fields of biochemistry, drug delivery and the most important in diagnosis and therapeutic applications. Loureirin B (LB) Luteolin and other hydrophobic marker compound of chinese herbal plants, which exhibits great potentials such as antimicrobial, anti-inflammatory, anti-cancer, antiradiation and antimutagen. However, its poor bioavailability, hydrophobicity, and pharmacokinetics restrict clinical application. Nano biomaterials synthesized by hot-micro emulsion ultrasonic, or thin film evaporation technique to improve the bioavailability & pharmacokinetics of compound. Synthesized nanoparticles size have been confirmed by particle size analyzer with range from 10 to 100 nm, having zeta potential -9.2 mV and polydisperse index 0.247, respectively. Drug encapsulation efficiency investigated by HPLC/Uv-Vis spectrometry. Both in vitro and vivo studies, LC-MS/MS technique was used for quantification of Loureirin B (LB) and Luteolin in rat plasma. The T_{max} value of drug with nanomaterials after the administration was Ten times shorter than pure Luteolin suspension administration. C_{max} value of drug after the administration of Nanomaterial with drug was five times higher than obtained with native drug suspension. In the end, the relative bioavailability of nanomaterials has improved about 4.89 compared to Loureirin B and Luteolin. From this study, it can be concluded that nanomaterials have not only great potential for improving solubility but also increased the drug concentration in plasma.

O.2.8. TECHNETIUM-99M LABELED IBUPROFEN: DEVELOPMENT AND BIOLOGICAL EVALUATION USING STERILE INFLAMMATION INDUCED MICE MODEL

Naeem-Ul-Haq Khan¹, Syed Ali Raza Naqvi¹, Samina Roohi²

¹Department of Chemistry, Government College University, Faisalabad-38000, Pakistan

²Isotope Production Division (IPD), Pakistan Institute of Nuclear Science and Technology (PINSTECH), Nilore, Islamabad-Pakistan

Corresponding author Email: naeemulhaqk@student.unimelb.edu.au

In this study we are presenting the development of technetium-99m (^{99m}Tc) labeled ibuprofen for the imaging of aseptic inflammation. ^{99m}Tc-Ibuprofen complex was developed by optimizing the radiolabeling conditions such as reaction time, ligand and reducing agent concentration, pH, reaction time and temperature. After addition of 600 µg of ibuprofen, 4 µg of stannous chloride as reducing agent and about 300 MBq ^{99m}Tc radioactivity, the mixture was allowed to react for 15 min at room temperature and 11 pH. Chromatography analysis revealed >94% ^{99m}Tc ibuprofen complex formation with promising stability in saline and blood serum up to 6 h. Biodistribution study using normal and sterile inflammation induced mice indicated low accumulation of labeled compound in major body organ, however, kidneys (14.76 ± 0.87 % ID/g organ) and bladder (31.6 ± 3.0 % ID/g organ) showed comparatively higher radioactivity due to principal excretory path. Inflammation tissue to normal tissue ratio (T/NT) at 1 h showed significant value (4.57 ± 0.56). Radiochemical purity of ^{99m}Tc-ibuprofen and biological evaluation using sterile inflammation induced mice revealed the agent bears promising potential to locate the aseptic inflammation which can be utilized to discriminate the septic and aseptic inflammation in combination with true septic inflammation agent.

O.2.9. THE PROACTIVE BIO-PHARMACOLOGICAL POTENTIALS OF PLEUROTUS SPECIES

Asma Irshad^{1*}, Muhammad Shahid², Muhammad Riaz³ and Zinayyera Subhani²

¹Center of excellence in Molecular Biology, University of the Punjab, Lahore-Pakistan

²Department of Biochemistry, University of Agriculture Faisalabad-Pakistan

³Department of Allied Health Sciences, Sargodha Medical College, University of Sargodha-Pakistan

Corresponding author E-mail: asmairshad76@yahoo.com

The edible mushrooms are highly useful for daily life, aimed at their biological and pharmacological potentials as they directly associated with the protection against various diseases influenced by life-style habits. The present study was conducted to evaluate the antioxidant, antimicrobial, nutraceutical activities of extracts of four *Pleurotus* spps. to fine out the biological and pharmacological potentials of given mushrooms. The extraction was performed by classical organic solvent extraction (COSE). The extracts of *Pleurotus* spps. were subjected to antioxidant activities analysis including TPC, TFC, DPPH and reducing power. The antimicrobial potential was studied against selected bacterial (*E. coli*, *P. multocida*, *B. subtilis* and *S. aureus*) and fungal species (*A. niger*, *A. flavus*, *F. solani* and *H. maydis*). Nutraceutical analysis including α -tocopherols and β -carotenes was performed by the HPLC. Extraction yields by (COSE) was found to be 5.7 to 76.4 g/100 grams (w/w) for all *Pleurotus* spps. The *Pleurotus* spps contained appreciable levels of TPC (0.95-19.49, mg/100g), TFC (0.85-3.73, mg/100g), DPPH radical scavenging activity (19.15-54.50 %) and reducing power (0.50-2.94 nm) respectively. A moderate antimicrobial potential was shown by all *Pleurotus* spps against selected bacterial and fungal species. Nutraceutical analysis expressed the HPLC analysis of α -tocopherols of four mushroom species as *P. sapidus*>*P. ostreatus*>*P. sajor-caju*>*P. columbinus* respectively and the HPLC analysis of β -carotenes was as *P. sapidus*>*P. columbinus*>*P. ostreatus*>*P. sajor-caju* respectively. The results of four *Pleurotus* spps. extracts showed that they can be used as an excellent medicinal mushroom in pharmaceutical and cosmetics industry. Furthermore, teamwork of biochemists and pharmacist is required to further improve its specificity and stability profile. Moreover, such conferences are excellent source of fruitful collaboration among the researchers.

Keywords: Antioxidants, antimicrobial agents, nutraceutical activities and *Pleurotus* spps.

**O.2.10. ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AGENTS
FROM THE EPIDERMAL MUCUS OF LABEO ROHITA AS AN ANTIMICROBIAL
DRUG FOR THE TREATMENT OF MICROBIAL INFECTIONS**

Hina Tabsum¹, Muhammad Anjum Zia¹, Zaheer Ahmed¹

¹Enzyme Biotechnology Laboratory (EBL), Department of Biochemistry,
University of Agriculture, Faisalabad, Punjab 38040, Pakistan.

Corresponding author Email: mbczaheer@hotmail.com

The epidermal mucus of fish species has been found to contain antimicrobial proteins and peptides, which is of interest in regard to fish immunity. *Labeo rohita* was collected from the wild site showed the highest activity against pathogens as compared to the control site. The mucus was tested for antibacterial activity against double pathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Pseudomonas putida*, and *Streptococcus equisimilis*. The maximum and minimum antibacterial zones of inhibition were exhibited by *Staphylococcus aureus* of the wild site (7.58 ± 0.081) and *Streptococcus equisimilis* of control site (3.2 ± 0.163). The mucus was tested for antifungal activity against double pathogenic strains of *Aspergillus terreus*, *Aspergillus niger*. Highest and lowest diameter of inhibition zones were showed up by *Aspergillus niger* of wild and *Aspergillus terreus* of control site that was (6.7 ± 0.163) and (5.7 ± 0.205) respectively. Estimation of proteins was also done for mucus from the river and intensive system, their results show that protein concentration was high (2.51 ± 0.139) in the control site than the river areas as (1.72 ± 0.198). Amino Acid analysis was done to identify the composition of amino acids in the mucus. Some important amino acids were found in the mucus of *Labeo rohita* at high percentages such as phenylalanine, proline and the moderate amount of lysine, ammonia, and aspartate.

Keywords: Identification, antimicrobial, *Labeo rohita*, mucus and drug

O.2.11. DETERMINATION OF ANTIBIOTIC RESISTANT GENES IN LACTOBACILLUS SPECIES ISOLATED FROM COMMERCIALY AVAILABLE FERMENTED YOGURT

Zunaira Hassan¹, Muhammad Ashraf¹, Muhammad Naeem Faisal^{2*}, Razia Kousar³, Rizwan Aslam¹ and Sultan Ali¹.

¹. Institute of Microbiology, University of Agriculture Faisalabad, Pakistan.

². Institute of pharmacy, Physiology and pharmacology, University of Agriculture Faisalabad, Pakistan.

³. Institute of Anatomy, University of Agriculture Faisalabad, Pakistan.

Corresponding author Email: Zunairahassan58@gmail.com

Fermented foods have great interest due to the presence of lactic acid bacteria i.e. Lactobacillus. That bacteria fall in probiotic category that has tremendous beneficial effects on gut health. Lactobacillus is permanent inhabitant as intestinal microbiota. The genus works as reservoir for resistance genes and also involves in dissemination of resistant genes to pathogenic strains. Antibiotic resistance can transfer through different mechanisms as horizontal gene transfer mechanism among lactic acid bacteria to other bacteria. Current study is designed to determine the availability of antibiotic resistance genes in isolated Lactobacillus from commercially available fermented yogurt that exhibited antibiotic resistance. The presence of antibiotic resistance genes was analyzed in Lactobacillus acidophilus. For this purpose commercially available fermented yogurt samples were used. Total 3 types of fermented yogurt samples were used. Samples were positive to Lactobacillus acidophilus. Different tests were applied for identification and resistance was detected by antibiotic susceptibility assay. Lactobacillus acidophilus was resistant to metronidazole, amoxicillin and ampicillin. Genomic DNA was isolated with the help of mini kit. Nano drop method was used for quantification of DNA. Amplification was done with PCR. Molecular confirmation was done by the visualization of PCR product in gel Electrophoresis. 1-7 bands were produced for gene of Lactobacillus acidophilus. Gene sequencing analysis of Lactobacillus acidophilus was done with the help of expression of three genes that were tet(M), erm(B) and sul(2) genes. Analysis was done by the comparison of two samples. Results showed that Lactobacillus acidophilus that was isolated from commercially available fermented yogurt contained minute resistance against sul(2) and tet(M) genes and also showed no resistance against erm(B) genes. According to statistical analysis this resistance was non-significant.

O.2.12. EVALUATION OF ANTIBACTERIAL PROPERTIES OF NANO-SIZED IRON OXIDE AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

Maria Zaib, Dr. Younas khattak and Dr. Bushra Arshad

Department of Biotechnology, Mohi- Ud-Din Islamic University, Nerian Sharif, AJ&K

Corresponding author Email: bushraarshad1988@gmail.com

Biotechnology and nanotechnology are two of the 21st century's most promising technologies. Nano-biotechnology is considered to be the unique fusion of biotechnology and nanotechnology and play a vital role in developing and implementing of many useful tools in improving life. However, the emergence of nanoparticle (NPs) technology has come with the promising broad range biomedical applications. A nanoparticle is the most fundamental component in the fabrication of a nanostructure. Iron oxides are one of the most important transition metal oxides of biomedical importance. Well-developed surface chemistry of Iron oxide nanoparticles (IONPs) provides an opportunity to incorporate a variety of biomolecules applications and also act as antibacterial agents. In current study, IONPs have been synthesized by co-precipitation method. The X-ray diffraction (XRD) analysis revealed that the synthesized IONPs (48 nm) are crystalline in nature. Mean particle size and morphology of synthesized IONPs was confirmed by SEM analysis which shows cubic spinel in shape. Furthermore, the antibacterial effect of IONPs at pre-defined concentrations 50, 100 and 150 mg/mL were evaluated against *S. aureus*, *S. flexneri*, *P. Aeruginosa*, *S. typhi*, *S. dysentery* and *E.coli*. Bacterial strains, which showed that the nanoparticles have potent antibacterial activity. Results revealed that iron oxide nanoparticles might have potential bactericidal activity and applicable in pharmaceutical and biomedical industries.

Keywords: Bactericidal activity, iron oxide nanoparticles, nanobiotechnology, X-ray diffraction and scanning electron microscopy.

O.2.13. EFFICACY OF NOVEL CHOLINESTERASE INHIBITORS AGAINST ALZHEIMER'S DISEASE AND RELATED DEMENTIAS

Hidayatullah Khan,

Departments of Chemistry, University of Science & Technology, Bannu

Corresponding author Email: hidayat_sks@yahoo.com

The aim of the cholinesterase inhibitors is to boost the endogenous levels of acetylcholine in the brain of Alzheimer's disease patients and herby, to boost cholinergic neurotransmission. Currently several cholinesterase inhibitors such as tacrine, revistagmine, donepizel and galanthamine has been used in the first mark for pharmacotherapy Alzheimer's disease. However these drugs have severe side effects like hepatotoxicity and gastrointestinal disorders. Hence there is a great interest in the discovery of novel AChE inhibitors from natural sources suh as huperzine, bacosdies, hyperforin used in the treatment of neurological disorders. New aconitine type C₁₉ nor diterpeniod alkaloid Isotelatazidine hydrate isolated from *Delphinium denudatum* Wall was tested for their acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) inhibitory activities in dose dependent manner. The IC₅₀ values of compounds against AChE and BChE were 11.13± 0.43μM and 21.41± 0.23μM respectively. atisine type C₂₀ diterpenoid alkaloid Ajaconine **1** and lycoctonine type C₁₉ Norditerpenoid alkaloid Delectinine **2** isolated from *Delphinium chitralense* were tested for their acetylcholinesterase (AChE) and btyrylcholinesterase (BChE) inhibitory activities in dosedependent manner. The IC₅₀ (50% inhibitory effect) values of compounds CD6 and CD8 against AChE were 5.04± 0.09 and 12.61 ± 0.05μmM while the values obtained against BChE were 9.21± 0.06 and 10.18± 0.91 μM respectively. All the nitrogen containing novel derivatives of progesterone (**1-16**) were screened for their acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities in dose dependent manner. The IC₅₀ (50% inhibitory effect) values of compounds **1**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, and **13** were showed strong activity against AChE as 14.40 ± 0.08, 18.02 ± 0.19, 19.08 ± 0.18, 23.11 ± 0.05, 21.47 ± 0.06, 22.18 ± 0.13, 20.27 ± 0.08, 15.97 ± 0.07 and 17.01 ± 0.02 μmM while the values obtained against BChE were 22.21 ± 0.06, 28.01 ± 0.01, 26.12 ± 0.09, 37.31 ± 0.11, 34.14 ± 0.05, 32.45 ± 0.20, 30.15 ± 0.16, 20.08 ± 0.01 and 30.44 ± 0.14 μM respectively. Compounds **11**, **16**, and **17** showed weak inhibition profile. All compounds were docked against AChE and BChE were showed most effective interaction. The synthesis of acetylcholine from choline and acetyl-coenzymeA (Acetyl-coA) in presynaptic neurons is catalysed by the enzyme cholineacetyltransferase. When released, acetylcholine's effects are mediated via pre- and post-synaptic muscarinic and nicotinic receptors. Results may be either excitatory or inhibitory. Released acetylcholine is broken down within the synaptic cleft by the enzyme acetylcholinesterase (AChE)

O.2.14. SYNTHESIS OF POLYMERIC NEXUS FOR TAREGETED DRUG DELIVERY AND ITS IN-VITRO EVALUATION

Farooq Azam*¹, Hira Ijaz¹

^{1*}Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

Polymers play potential role in the development of drug-delivery systems. This research work investigated the use of Hydroxyethyl cellulose (HEC), acrylic acid (AA) and N'N'-methylene bis-acrylamide (MBA) in the synthesis of hydrogels for controlled drug delivery of acid sensitive drug perindopril erbumine (PE). Different ratio of HEC, AA and MBA were blended with each other to fabricate hydrogels via free radical polymerization technique. Fourier transform infrared spectroscopy (FTIR) revealed successful grafting of components into the polymeric network. Thermal and morphological characterization confirmed the formation of thermodynamically stable hydrogels having porous structure. The pH responsive behavior of hydrogels has been documented by swelling dynamics and drug release behavior in simulated gastrointestinal fluids. Drug release kinetics revealed controlled release behavior of the perindopril erbumine in developed polymeric network. Cross-linked Hydroxyethyl cellulose (HEC), hydrogels can be used as promising candidates for the design and development of controlled drug-delivery systems.

Keywords: Hydroxyethyl cellulose (HEC); acrylic acid (AA); Hydrogel; pH-Responsive

O.2.15. SERUM AND CELLULAR PHARMACOKINETICS OF SOFOSBUVIR AND ITS CONCENTRATION EFFECT ANALYSIS IN HCV PATIENTS RECEIVING SOFOSBUVIR AND RIBAVIRIN THERAPY

Saba Shehzadi, Bilal Ahmed, Sara Ashraf, Aqsa Afzal, Atika Nageen, Bushra Munir and

Abdul Ghaffar

Department of Biochemistry, Government College University, Faisalabad, 38000, Pakistan

Corresponding author Email: aghaffaruaf@yahoo.com

HCV has been major cause of death for decades in Pakistan due to poor health conditions and management. Sofosbuvir and ribavirin have emerged as direct acting antivirals agents for the proper eradication of viral load in HCV patients. The present study reports serum and cellular pharmacokinetics and concentration effect analysis of sofosbuvir in human. HCV positive individuals and receiving 400 mg sofosbuvir along with low dose or weight base ribavirin were used in this study. Concentrations were modeled through NONMEM and unpaired t-test was used for the association of concentrations and treatment outcomes. Average day 10 sofosbuvir metabolite BM 331007 concentration was higher in patients having haemoglobin nadir <10 g/dl with relative to the patients having haemoglobin >10 g/dl (5.34 versus 4.87 pmol/ 10^6 cells; $p=0.03$). the average concentration trends of GS331007 at day 10 was towards being higher in the patients which achieves sustained virologic response (SVR) as compare to the patients who relapsed (5.19 versus 4.86 pmol/ 10^6 cells; $p=0.05$). Sofosbuvir (GS331007) thresholds concentration which was suggested at day 10 through receiver operating characteristic curve was 5.4 pmol/ 10^6 cells for SVR ($p=0.05$) and haemoglobin nadir cells was 6.3 pmol/ 10^6 with sensitivity and specificity of $\geq 60\%$. Dosing simulations shows that 400 mg sofosbuvir twice daily produce day 10 concentration range of 5.4 to 6.7 pmol/ 10^6 cells. It was concluded that GS 331007 concentration in serum at day 10 was related to the SVR and Anemia. The range of therapeutic values was identified for HCV patients receiving sofosbuvir plus ribavirin for 24 weeks, suggesting a potential pharmaceutical basis for individualized therapeutic dosing.

Keywords: Hepatitis C virus (HCV), Sustained virologic response (SVR), GS 331007

O.2.16. PHYTOPHARMACEUTICALS: PROMISING CANDIDATE FOR DISEASE PREVENTION IN HUMAN

Zeshan Zulfiqar*, Shahid Ur Rehman, Sidra Bashir, Muhammad Khalid Bashir, Umar Farooq, Umair Mahmood, Mariam Zehra, Muhammad Farooq Khalid
University of Agriculture Faisalabad, Sub Campus Toba Tek Singh
Centre for Applied Molecular Biology & Forensic Sciences University of the Punjab
Corresponding author Email: 4zeshan@gmail.com

Plants are gift of nature. These are used as food, increase nature beauty and also used for medicinal purposes. As the diseases are emerging day by day there is also need to cure them. Nature has provided us tremendous therapeutic agents hidden in plants which we have to explore. Herbs are plants or plant parts that are used as medicine and contain chemical compounds that act upon the body and are related to different diseases. Many plants are known so far e.g. Cichorium intybus is used as nerve tonic, Anthemis nobilis as anti-inflammatory and anticoagulant, Papaver orientale as analgesic, Alhagi for wound healing and kidney stones, Echeveria elegans for headache, Paliurus spina for magarine, Alcea aucheri for preventing hair loss, Ziziphus jujuba for cold treatment. Medicinal plants are also useful for treating chronic diseases such as cancer e.g. Celosia trigyna contains ethanol that cytotoxic activity against cancer and promotes apoptosis, Cannabis sativa contains methyl alcohol that exert antitumor effect by inducing apoptosis and also decreases telomerase activity by hindering TERT gene expression. Mangifera indica contain Lupeol that act as cure for skin cancer. Medicinal plants have advantage over conventional plants as they are safe and less costly. These are derived from nature and have less side effects as compared to conventional plants. Thousands of plants are known but still many are unknown to us. Further study should be done in this regard to discover plants worldwide and exploring their medicinal value for medical uses and for disease treatment in less costly manner.

Keywords: Herbs, TERT gene, nerve tonic, therapeutic agents

O.2.17. ANTI-DIABETIC ACTIVITY AND SYNTHESIS OF BENZIMIDAZOLE-BENZOTHAZINE STRUCTURAL HYBRIDS

Matloob Ahmad^{1,*}, Afshan Kanwal¹, Sana Aslam², Usman Ali Ashfaq³

¹Department of Chemistry, ²Department of Chemistry, ³Department of Bioinformatics and Biotechnology, GCUF, Pakistan.

Corresponding author Email: Matloob.Ahmad@gcuf.edu.pk

In this work, a novel series of benzimidazole-benzothiazine based Schiff bases were synthesized. The starting precursor methyl 2-(1,1-dioxido-3-oxobenzo[d]isothiazol-2(3H)-yl)acetate was synthesized by the coupling of sodium saccharine and methyl chloroacetate, which was rearranged to methyl 4-hydroxy-2H-benzo[e][1,2]thiazine-3-carboxylate 1,1-dioxide under basic conditions. The product was hybridized with benzimidazole ring system and followed by the reaction with hydrazine monohydrate to obtain 2-((1H-benzo [d]imidazol-2-yl))-4-hydroxy-2H- [1,2]benzothiazine-3-carbohydrazide 1,1-dioxide. The targeted benzimidazole-benzothiazine based Schiff bases were obtained by the condensation of hydrazide with various substituted aromatic aldehydes and ketones. All the synthesized compounds were characterized by spectroscopic techniques like MS, IR and NMR. The synthesized compounds were screened for their antidiabetic activity which resulted in the discovery of excellent bioactive compounds.

O.2.18. SYNTHESIS OF AG (I)-N HETEROCYCLIC CARBENE COMPLEXES FOR ANTICANCER STUDIES

Aqsa Habib*, Haq Nawaz Bhatti, Muhammad Adnan Iqbal

Department of Chemistry, University of Agriculture Faisalabad-38040, Punjab, Pakistan

Corresponding author Email: aqsahabib_uaf@yahoo.com; hnbhatti2005@yahoo.com

Silver complexes have become attractive tool in the field of medicinal chemistry. The present project was designed to synthesize new series of silver-*N* heterocyclic carbene (NHC) complexes. Various NHCs precursors were designed leading to the syntheses of target silver complexes, respectively. Three series of novel mono- and bis-imidazolium salts as ligands and their mono, di, tri and polynuclear silver adducts were synthesized. The corresponding Ag(I) adducts were afforded by the *in situ* deprotonation of the NHC ligands. All the precursors and silver complexes were well characterized by FTIR, GC-MS, ^1H , ^{13}C (one and two dimensional), ^{31}P and ^{19}F NMR, elemental analysis, melting points, ESI-MS and X-ray crystallographic techniques. After successful syntheses, the silver complexes were employed to study their anticancer activities against EAhy, MDA, A549 and Hella cancer cell lines. All the salts and silver complexes showed potential anticancer activity against tested cancer cell lines. The complexes were found to have many fold better results compared to the respective salts concluding that the silver imparts greater anticancer potential to the compounds. On the whole the present project provided useful information for drug designing.

Keywords: Silver Adducts; Heterocyclic carbenes; Biological activity; Anticancer Activity

**O.2.19. INVESTIGATION OF ERYPTOTIC ACTIVITY OF METHOTREXATE AND
ANTI ERYPTOTIC POTENTIAL OF AQUEOUS EXTRACTS OF *CURCUMA
LONGA* AND *ALLIUM SATIVUM***

Tayyba Sattar and Kashif Jilani

Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Erythrocytes are cells that contain hemoglobin and survive for only 3-4 months. Erythrocytes are anucleated and lack mitochondria. Eryptosis is the suicidal death of RBCs. Main features are cell shrinkage, cell membrane destruction, energy shortage and phosphatidylserine translocation. Eryptosis stimulated by oxidative stress, activation of kinases and elevated intracellular calcium level. Folate antagonist methotrexate is an anti-cancerous and anti-inflammatory agent that inhibit the folate dependent enzymes. This chemotherapeutic agent has ability to induce eryptosis and oxidative stress. Erythrocytes were treated with different physiological doses of methotrexate and decline in antioxidative defensive system was observed. Oxidative stress is the leading cause of cell death. Perennial herb turmeric is extensively cultivated in Asia. The rhizome is used medicinally gives up yellow powder. Source of turmeric is dried *Curcuma longa* that gives yellow color. It is traditional medication used against many ailments like gastric ulcer, jaundice, skin diseases, and joint inflammation. *Allium sativum* is broadly cultivated antioxidative plant for its therapeutic purposes. Erythrocytes were treated with *Curcuma longa* and *Allium sativum* aqueous extracts to check the anti oxidation and anti eryptotic potential of both plants. It is observed that the toxic effects of methotrexate were neutralized by aqueous extracts of used plant.

Keywords: Methotrexate, Extracts, Eryptosis

O.2.20. RENAL CLEARANCE AND URINARY EXCRETION OF IBUPROFEN IN MALE VOLUNTEERS

Mubashra Khan and Tahira Iqbal

Department of Biochemistry, University of Agriculture Faisalabad, Pakistan

Corresponding author Email: mubashrakhan04@gmail.com

Ibuprofen is an anti-inflammatory, antipyretic and analgesic drug. It is commonly used to get relief from pain and fever. It can be used for various diseases like Alzheimer's, orthodontic disorders, cystic fibrosis of lungs etc. It works by inhibiting the production of prostaglandin. Excretion through urine is the primary way for the elimination of drug from the body, so, the urinary excretion and renal clearance of ibuprofen was determined by taking blood and urine samples from male volunteers after the administration of ibuprofen tablets. Before the administration of ibuprofen, blank blood and urine samples was taken. Volume and pH of urine samples will be determined and recorded. Blood and urine samples was analyzed by HPLC to determine the concentration of ibuprofen. Standard curve of ibuprofen was prepared by using regression analysis and concentration of ibuprofen in samples was determined by using regression equation.

Keywords: Ibuprofen, Urinary extraction, Renal clearance, Male volunteers, HPLC

TRACK 3

**MOLECULAR BIOLOGY, GENETIC
ENGINEERING AND BIOINFORMATICS**

TRACK COMMITTEE

- 1. PROF. AMER JAMIL (CONVENER)**
- 2. DR. AFTAB AHMED**
- 3. DR. MUHAMMAD JAMSHED**
- 4. DR. ZAIN UL ABDIN**

KEYNOTE LECTURES

TRACK 3

K-3.1 ANIMAL BIOTECHNOLOGY & GENOMIC RESEARCH IN PAKISTAN; CURRENT SCENARIO & FUTURE PROSPECTS

Masroor Ellahi Babar, Akhtar Ali, Tanveer Hussain

Faculty of Science & Technology, Virtual University of Pakistan, Lahore

Animal genetic resources are very diverse in Pakistan. Animal resources are goat (74.1 million), Sheep (30.5 million), Cattle (46.1 million), Buffalo (38.8 million) and Camel (1.1 million). Genomic techniques used for investigation include sequencing, genotyping and microarray based genotyping. We have characterized indigenous animal breeds using FAO and ISAG molecular markers on local cattle, buffalo, sheep, goat, camel, yak, horses and chicken. We developed DNA bank ($\approx 10,000$ samples) of local species of Pakistan including domestic animals, wild animals and birds. Wild animals are Urial, Mouflon sheep, Markhor, Hog Deer, Chinkara, Black Buck while birds are domestic chickens, Pheasant, Peafowl, Chukar etc. Our findings include genetic characterization (Mt D-loop, Cyto b, COX1, ATPase6-8, FAO/ISAG rec. microsatellite markers), production traits (Meat, Milk, Fertility), disease resistance genes (MHC Complex genes, ILs, DRBs), heat shock protein genes (Hsp40,70,90), barcoding of endangered species in Pakistan (COX1), prion genes in domestic and wild animals (Prp). Now we are heading towards NGS, GBS, ddRAD, Transcriptome analyses with international collaborations.

K-3.2 JOURNEY OF DNA EVIDENCE FROM GENOTYPING TO PHENOTYPING

Nouman Rasool

Department of Life Sciences, University of Management and Technology, Lahore.

The recent development in the field of forensic DNA analysis cast a huge impact on criminal justice systems around the globe. This technique is playing pivotal role in solving crimes by convicting the criminals without any doubt. The courts are relying much on Forensic DNA reports than eyewitnesses during trial while deciding cases. The analysis carried out by determining the genotypes of evidence and reference samples. For comparison with evidence samples, the reference DNA profiles are required to made inclusion or exclusion of perpetrator in a crime. In case of non availability of such profile, it becomes hard to identify suspects. With advent of new technologies, phenotypic analysis of DNA samples can be carried out which includes various physical characteristics of an individuals. Today we are able to develop gender, skin color, iris color, facial features and gait of the perpetrator using this technology. Modern way to perform DNA sequencing has revolutionized such identifications. It means that when someone deposits its DNA at crime scene during commission of a crime, actually he is depositing his photograph there. These technological advancements will help in reducing crime in society and provide speedy justice to everyone.

ORAL PRESENTATIONS

TRACK 3

O.3.1. PARTIAL CHARACTERIZATION OF EXTRA-CHROMOSOMAL DNA FROM MULTIDRUG RESISTANCE STAPHYLOCOCCUS SPECIES OF LOCAL ISOLATES PRESENT IN THE NASAL FLORA CHILDREN UNDER FIVE YEAR OF AGE

Amtul Jamil Sami^{1*}, Madeeha Khalid¹, Sermad Ahmad Mangat²
and Hammad Ahmad Mangat³.

¹Institute of Biochemistry and Biotechnology, University of the Punjab, Quaid-e-Azam campus, Lahore 54590, ²Pakistan. (2) CMH-Medial College Lahore Abdur Rahman Road Lahore Pakistan. ³Faryal Dental College Lahore Motorway. Shiekhupura.

Corresponding author Email: amtuljamisami@gmail.com

Pneumonia is one of the major killer diseases among children of age <05 years. In Pakistan, the quantification of the burden of pneumococcal disease through surveillance has not been focused thus, the problem is being unaddressed. Immunization against pneumococcal disease could help to attain WHO Millennium Development Goal, in Pakistan. Methods: We designed a study to estimate the burden of invasive pneumococcal diseases and antibiotic-resistance patterns of Staphylococcus Species isolates in children aged <5 years in Lahore, Pakistan. To meet the objectives children belong to affluent background and camp cities were studied. Nasal swabs were collected from the subjects and the microbial flora was identified. All the subjects had in their nasal flora. The Staphylococcus strains were tested for drug resistance using a number of antibiotics. Results: It was found 20% of the subjects (effluent background) had resistant strains of Staphylococcus Species in their nasal flora, whereas over 50% resistance was observed in the samples of camp city children. The presence of an extra chromosomal DNA of phage origin was recorded, in the resistant strains. The resistant bacterial was also identified in the drinking water samples of the area. The extra-chromosomal DNA was partially sequenced and analyzed. It was concluded that the major role in the spread of epidemic of Staphylococcus Species disease could be viral. The extra chromosomal DNA identified can be a major contributor to pathogenesis and strain diversity via horizontal gene transfer (HGT).

Keywords: Pneumonia, Staphylococcus, drug resistance, extra chromosomal DNA

O.3.2. PROTEOMIC ANALYSIS OF A TRUNK DISEASE OF FRUITING TREES CAUSED BY A NOTORIOUS FUNGAL PATHOGEN

Tanveer Hussain^{1*}, Muhammad Ishtiaq¹, Tony Adesemoye² and Sumaira Thind³

¹Department of Botany, Mirpur University of Science and Technology (MUST) Mirpur, Azad Jammu & Kashmir ²Department of Plant Pathology, WCREC North Platte, University of Nebraska, Lincoln, USA

³Department of Botany, Government College University Faisalabad-38000-Pakistan

Corresponding author Email: tanveerajk@gmail.com

Attack of fungal pathogens on fruiting trees is a big problem in many important plants worldwide. A trunk disease caused by a fungus “*Lasioidiplodia theobromae*” is very epidemic in fruiting trees. Pathologists try to check the interaction of the pathogen with host with special reference to protein functions. Proteomics is a highly useful technology for studying protein expression and for discovering new proteins in any host. The recent proteomics approaches used for protein extraction of the fungus were SDS-PAGE (2-D) and multiple technologies including protein mass spectrometry, sequencing and advances in bioinformatics. Yet, the assignment of function of fungal proteins encoded as *in silico* annotated or un-annotated genes. It is still remains problematic. The main aim of the analysis is to find out the unknown function of protein. A combination of approaches including comparative proteomics, pathogen-induced protein expression and immune-proteomics are used in combination with other techniques to explore the comprehensive information on protein function of the specific fungi. These proteins and their functions may be targeted for further functional characterization and validation efforts due to their potential uses in fungal biotechnology as well as various novel proteases. This work contributes to genomic annotation efforts which explicated genomic sequencing of the specific fungi. It will help to improve fungal bioinformatics databases for future studies of different fungal pathogens. In future, quantitative proteomics, combined high-throughput sequencing and transcriptomic sequencing of protein function in different fungal species are set goal of pathologists.

Keywords: Fungal pathogens; Fruiting trees; Fungal proteomics; Genomic sequencing; Multiple technologies; Bioinformatics databases

O.3.3. A BIOINFORMATIC STUDY OF LIPASES FROM REPORTED THERMOPHILIC BACTERIA

Rozina Aslam¹ and Muhammad Sarwar Yaqub²

¹Department of Chemistry; ²Department of Horticultural Sciences,

The Islamia University of Bahawalpur, Pakistan

Corresponding author Email: hlb92@yahoo.com

The objective of this bioinformatic study was to analyze lipases, reported from thermophilic bacteria. Lipase sequences were recovered from Uniprot KB/SwissProt. The unfiltered aggregate of lipase sequences from different species including fungi, archaea, and bacteria were 1056. After removing lipase sequences of fungi and archaea, 511 sequences left for bacteria. Uncharacterized proteins, regulatory proteins, Precursors, hydrolases, peptides, carrier proteins and proteins which could not be classified on the basis of temperature were eliminated. After elimination of non reviewed sequences, five sequences from thermophilic bacteria were left and studied comparatively. Physico-chemical properties were compared using Protparam and Protscale software. SOPMA and GOR IV software were used for the analysis of secondary structure composition. GOLDMAN PRANK (Probabilistic Kit) and Jalview were used for multiple sequence alignment. The presence of conserved domains were studied using CD-BLAST, whereas Phyre2 was utilized for predicting 3D-structures. 3D-Ligandsite online server was used for finding ligand binding sites. ProtParam analysis showed that lipases from thermophilic bacteria had high percentage of valine (V), proline (P) and glutamic acid (E) residues. ProtScale analysis indicated that heat resistant lipases had higher percentage of polar charged residues, C_vP indices, IVYWREL, and Alpha helix content. Sequences aligned by Goldman Prank gives higher matching score between basic and hydrophobic residues suggesting that, replacement of acidic residues with basic residues may increase thermostability of lipases.

Keywords: Goldman Prank, Protparam, Protscale, SOPMA, Phyre2

O.3.4. CHARACTERIZATION OF LARVICIDAL ACTIVITY OF BIOGENIC NANOPARTICLES

Neeha Ghafoor¹, Sheeraz Ahmad¹, M Javaid Asad¹, Kumail Ali Rizvi², Tayyaba Zainab¹

1. University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah -Arid Agriculture University Rawalpindi.

Corresponding author Email: neehaghafoor@gmail.com

Synthesis of green nanoparticles (NPs) is a one of the most emerging field of Nano biotechnology. Plant extracts are used for the synthesis of green NPs. Green silver nanoparticles are used as vector mosquito control agent against *Aedes aegypti*, vector of Dengue. Silver NPs are more effective than Gold or any other metal because they have property of high stability. AgNO₃ solution was mixed with appropriate amount of Crude Methanolic Extracts (CME) of *Eucalyptus globulus* and *Curcuma longa* to form green AgNps with vigorous shaking. Characterization of these green AgNps is done with U/Visible Spectroscopy for confirmation of silver nanoparticles and Scanning Electron Microscope (SEM) to analyze the nanoparticle size and shape. Both samples showed absorbance between 420-430nm. SEM results showed spherical shape of green silver nanoparticles. The size of nanoparticles of *Eucalyptus globulus* is approximately is 25nm and the nanoparticles of *Curcuma Longa* are of 15nm. These Green NPs were proved to be very effective against 3rd and 4th larval stage of *Aedes aegypti* as they are eco-friendly, less harmful and have strong larvicidal activity. Overall AgNPs of both plants exhibited significant larvicidal activity.

Keywords: Green synthesis, Nanoparticles, Dengue, Larvicidal, Characterization, Silver

O.3.5. IN-SILICO PREDICTION OF ZIKA VIRUS EPITOPES AGAINST BRAZILIAN HUMAN LEUKOCYTE ANTIGEN BACKGROUND: AN APPROACH TOWARDS DEVELOPMENT OF FUTURE VACCINES FOR BRAZILIAN POPULATION

Malik Siddique Mahmood^{1*}, Naeem Mehmood Ashraf², Aadil Hussain², Muhammad Zubair Mahmood², Nadia Zeeshan², Mahjabeen Saleem¹

¹Institute of Biochemistry and Biotechnology, University of the Punjab, Pakistan.

²Department of Biochemistry and Biotechnology, University of Gujrat, Pakistan.

Increasing infection of Zika virus threatening the infant's life by causing microcephaly in developing countries. Numbers of various approaches have been anticipated to develop a vaccine against Zika virus, but the majority of them proved ineffective. The potency of epitope-based vaccines is directly linked with genotypes of host genetics. In this research article, we have tried to predict most putative Zika Virus epitopes which are efficiently restricted by most common HLA alleles in Brazilian population through different computational algorithms. Databases allowed us to develop the consensus sequences from the highly-conserved proteins of Zika Virus strains reported from the Brazil. Obtained consensus sequences were used to predict their binding affinities with most prevalent HLA alleles in the Brazilian population. Two Class-I epitopes from E1 region, three from Class-I epitope from NS5, one Class-II epitope from E1 region and one Class-II epitope from NS5 region showed effective binding and proved to be highly putative to boost immune response. A cocktail of these seven has been checked for population coverage and they gave 99.99% for Brazilian populations with no allergenic response. Computational algorithms are robust way to shortlist potential candidate epitopes for vaccine development, but further, in vivo and in-vitro studies are required to confirm their immunogenic properties.

**O.3.6. CELL FREE CIRCULATING DNA QUANTIFICATION AND
METHYLATION STATUS OF DCC GENE AS PREDICTIVE DIAGNOSTIC
BIOMARKERS OF LUNG CANCER**

Muhammad Shahbaz Aslam, Abeera Shaeer, Iram Gull, Imran Tipu, Zaigham Abbas*,

Muhammad Amin Athar

Institute of Biochemistry & Biotechnology, University of the Punjab, Lahore

*Department of Microbiology & Molecular Genetics, University of the Punjab, Lahore

The worldwide high mortality rate of lung cancer could be reduced significantly by its non-invasive early detection. The quantitative analysis of cell free circulating DNA (cfDNA) in plasma presents a potential noninvasive approach for liquid biopsy of tumor. In this study, real time PCR based approach was used to quantify cfDNA in plasma. The concentration of cfDNA was checked using *hTERT* (human telomerase reverse transcriptase gene) as marker and amplification status of oncogene *AKT2* (RAC-beta serine/threonine-protein kinase) along with the DNA methylation status of tumor suppressor gene *DCC* (Deleted in Colorectal Cancer) was assessed. The concentration of cfDNA in lung cancer patients (22.8 ng/mL) was found approximately six times above than the value detected in controls (2.8 ng/mL). Considerable variation in the *AKT2* copy number was observed in lung cancer patients and controls ($p < 0.000$). Aberrant methylation of the *DCC* promoter was found to be highly specific (100%) as none of the control plasma samples showed aberrant methylation but was less sensitive (55.88%) ($p < 0.001$). The quantification of cfDNA along with determination of *AKT2* amplification and *DCC* promoter methylation status appears promising to differentiate lung cancer patients from healthy individuals.

O.3.7. PURIFICATION, N-TERMINAL AND PHYLOGENETIC STUDIES OF POLYPHENOL OXIDASE ISOFORMS FROM APPLE, PEAR AND BANANA FRUIT PULP

Naila Sajjad

PMAS Arid Agriculture University Rawalpindi

Corresponding author Email: nailasajjad1288@yahoo.com

Polyphenol oxidases (PPOs) are a group of copper containing enzymes. PPOs catalyze the oxidation of phenols to *o*-quinones that are highly reactive molecules can easily go through non-enzymatic secondary reactions to form brown complex polymers called melanins. The interaction with pests and pathogen and handling during storage and postharvest processing, wounding and senescence cause the loss of sub-cellular compartmentalization that ultimately results in contact between PPOs and vacuolar phenolic substrates. This overall process eventually leads to a reaction called enzymatic browning. It negatively affecting nutritional properties, flavor, color and shelf life of food products. Enzymatic browning might be useful in some cases as it ultimately leads to the formation of compounds conferring characteristic flavors. Polyphenol oxidase in plants is known to exist in many isoforms with different molecular weight. Currently, PPO attained important attention because it has high capacity for oxidizing aromatic compounds. This feature makes the use of PPO good for biotechnological applications in medicines, environmental technology, pulp and paper industry, food industry and textile industry. Keeping in view the significance of PPO in various fields the given study was designed to extract and comparatively analyze the PPO from apple, pear and banana fruit pulp. PPOs were extracted from fresh apple, pear and banana fruits after peeling and were purified by gel filtration chromatography. Two isoforms from apple having molecular weight (45kDa, 28kDa), three isoforms from banana (65kDa, 45kDa, 28kDa) and single PPO from pear (60kDa) were detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis. Purified PPOs were comparatively analyzed for pH, temperature, metal ions and enzyme kinetics. The maximum PPO activity for apple, pear and banana was found at pH 6.5. Optimum temperature for PPO activity was determined to be 30-40°C. Michaelis constant (K_m) and maximum reaction velocity (V_{max}) values using catechol substrate under optimal conditions were determined by means of Lineweaver-burk plot for apple, banana and pear. K_m and V_{max} values for apple (74.21mM, 4.45 μ M/ml) banana (53.33mM, 9.15 μ M/ml) pear (72.27mM, 2.94 μ M/ml) were calculated. Western blotting (Electroblotting) was used to transfer all the

proteins from the gel to an inert membrane (PVDF). N-terminal microsequencing of PPO isoforms from apple, pear and banana was performed on an applied biosystem pulse liquid protein sequencer for N-terminal protein sequencing. N-terminal microsequencing of 28 kDa PPO isoform from apple (Alanine, Lysine, Isoleucine, Threonine, Phenylalanine, Histidine, Glycine), 45 kDa PPO isoform from apple (Alanine, Proline, Glycine, Glycine, Glycine) 28 kDa isoform of banana PPO shows (Alanine, Proline, Asparagine, Serine, Tyrosine) and 45 kDa isoform shows (Alanine, Proline, Isoleucine, Alanine, Proline) and 60 kDa pear PPO shows (Serine, Leucine, Proline, Asparagine, Proline, Proline) amino acid residues. Multiple sequence alignment was performed by using the protein sequence from the gene data bank and sequence homology was studied by using CLASTALW tool. Phylogenetic tree of N-terminal sequence of the reported PPO isoforms in the given study shows strong resemblance with the protein having accession no AHA91117.1, AHA91116.1, AHH92831.1 and ACJ65307.1. PPO Protein contains three conserved regions: an N-terminal region, CuA, CuB (tyrosinase) domain and a C-terminal extension. N-terminal transit peptides of PPOs cleaved at an alanine motif after import into the thylakoid lumen. CuA and CuB domain contain several conserved histidine residues. The C-terminal end of the protein consists of DWL (Pfam 12142) and KFDV (pfam12143) domains.

**O.3.8. DEVELOPMENT OF EFFICIENT MATHEMATICAL MODEL FOR
OPTIMIZED BACTERIOCIN PRODUCTION FROM LOCAL ISOLATE OF
LACTOBACILLUS ACIDOPHILUS MS1 USING RESPONSE SURFACE
METHODOLOGY**

Mahwish Salman^{1*}, Anum Tariq¹, Misbah Shahid¹.

¹Department of Biochemistry, Government College University, Faisalabad-38030, Pakistan

Corresponding author Email: mahwishsalman@gcuf.edu.pk

Bacteriocins are antimicrobial peptides synthesized by bacteria and these are well known to control pathogenic microbial populations in fermented foods and extend their shelf-life and safety. The optimized production of bacteriocin was predicted by using Box-Behnken experimental design. The produced bacteriocin yields 2600AU/mL activity units at 30°C temperature, 6 initial pH and 18 h incubation time. The results derived from response surface methodology (RSM) regression equation indicated that the initial pH of the model system influencing stronger than other physical variables at 95% confidence level. Moreover, quadratic and interaction effects of these variables were also found significant. A monomeric peptide of 6.5 kDa was purified to 59.1-fold from crude sample using ion exchange and gel filtration chromatography. Purified peptide showed strong inhibitory activity against biofilm produced by *Bacillus cereus*, a food borne pathogen. Conclusively, Box-Behnken design can be used for large scale production of bacteriocin from locally isolated strain of *Lactobacillus acidophilus* MS1 that might be used as a preservative and non- allergic antibiotic in food as well as in pharmaceuticals.

Keywords: *Lactobacillus acidophilus*, bacteriocin, response surface methodology, Box-Behnken experimental design

O.3.9. PHYLOGENY AND AN *IN SILICO* STUDY TO REVEAL ALL POTENTIAL AND TOLERATED SINGLE-SITE MUTATIONS WITH INCREASED STABILITY OF STREPTOKINASE C

Rawaba Arif¹, Muhammad Anjum Zia and Ghulam Mustafa^{2*}

¹Department of Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan

²Department of Biochemistry, Government College University, Faisalabad-38060, Pakistan

Corresponding author Email: drghulammustafa@gcuf.edu.pk

Streptokinase which is also known as fibrinolysin helps in breakdown of fibrin that is the main component of blood clots. Various strains of β -hemolytic *streptococci* have been found to produce streptokinase. Streptokinase has been preferred over all thrombolytic agents as it is one of the less expensive and suitable among all. Since the discovery of streptokinase in 1930s, scientists have been working on developing mutant strains to hyper-express streptokinase. In this study, the bioinformatics tools I-Mutant and SIFT were used to explore all possible mutations that would be tolerated and involved in an increased stability of streptokinase. The sequence of streptokinase c from *Streptococcus dysgalactiae* subsp. *equisimilis* was taken as a query and all positive mutations were predicted in signal peptide and non-cytoplasmic domain of the enzyme. CASTp was used to find active site in streptokinase. Total 46 potential point mutations were found in active site at 9 different positions. All these mutations are involved in increased stability but not tolerated. Similarly, 19 potential point mutations at 2 different positions in signal peptide and 304 potential single-site mutations at 54 different positions in non-cytoplasmic domain of streptokinase were predicted. The mutations of both regions are involved in an increased stability of the enzyme and all are also tolerated. The homologs of streptokinase with $\geq 90\%$ identity were retrieved from UniProtKB and compared through multiple sequence alignment. A phylogram of query streptokinase and its homologous proteins was also generated to reveal their evolutionary relationships. The phylogram exhibited that query sequence has close evolutionary relationships with kinases, streptokinases and some hypothetical proteins from various *Streptococcus* spp. and *Escherichia coli*. The study will help scientists to find potential sites of mutations which could be tolerated to develop strains with increased stability of streptokinase. Further, these strains would be used to hyper-express streptokinase.

Keywords: Streptokinase; *Streptococcus dysgalactiae*; Single-site mutations; Phylogeny

O.3.10. MOLECULAR CHARACTERIZATION OF ATCNGC19 AND ATCNGC20 IN ARABIDOPSIS THALIANA FOR THEIR POTENTIAL ROLE IN SALT STRESS

Sadaf Oranab¹, Bushra Munir¹, Zulqurnain Khan², Sultan Habibullah Khan², Abdul Ghaffar^{1*}
and Aftab Ahmad^{2,3*}

¹Department of Biochemistry, Government College University, Faisalabad, Pakistan

²US-Pakistan Center for Advanced Studies in Agriculture and Food Security, University of Agriculture, Faisalabad, Pakistan

³Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: aftab.ahmad@uaf.edu.pk

We have previously reported finding of AtMIPS and AtbHLH106 for salt tolerance by activation-tagging of Arabidopsis calli (ICBBB 2016). Here we show identification and characterization of Arabidopsis thaliana Cyclic Nucleotide Gated Channel 19 (AtCNGC19) and Arabidopsis thaliana Cyclic Nucleotide Gated Channel 20 (AtCNGC20) in one of the mutants stc13 through activation tagging. In stc13, two insertions were confirmed on chromosome number 3 through TAIL PCR and in real time PCR, expression of AtCNGC19 was activated twice in control and about 5 times under 150mM NaCl stress level. AtCNGC20 was also present adjacent to AtCNGC19 on the same chromosome. Homozygous knock-out lines for AtCNGC19 and AtCNGC20 were generated by self-pollination, but both KO lines did not show any salt sensitivity in plants, however expression of both genes was significantly reduced in KO lines. Overexpression lines generated for both genes by expressing AtCNGC19 and AtCNGC20 under 35S constitutive promoter. The overexpression calli showed salt tolerance at 150mM NaCl compared with control calli however, when overexpression plants were exposed to different levels of NaCl, they did not show any tolerance. Similar results have been reported by other scientists but both genes show significant enhancement under salt stress in microarray there we assume that both genes compensate functions of each other during KO analysis. So, we further constructed CRISPR/Cas vector for simultaneous KO of both genes in same plant. Moreover, we have transformed overexpression lines of AtCNGC19 with 35S:AtCNGC20 construct to generate double overexpression lines. These results provide new evidences for role of AtCNGC19 and AtCNGC20 under salt stress regulation in Arabidopsis.

**O.3.11. AGROBACTERIUM MEDIATED DELIVERY OF MULTIPLEX
CRISPR/CAS9 SYSTEM IN COTTON AGAINST THE COTTON LEAF CURL
VIRUS DISEASE**

Komal Javed², Muhammad Zubair Ghouri ^{1,3}, Sultan Habibullah Khan^{1,3}, Zulqurnain Khan⁴,
and Aftab Ahmad.

¹Center of Advanced Studies in Agriculture and Food Security (CAS-AFS), UAF.

²Department of Biochemistry, ³Center for Agricultural Biochemistry and Biotechnology,
University of Agriculture, Faisalabad.

⁴Institute of Plant Breeding and Biotechnology, MNSUA, Multan

Corresponding author Email: aftab.ahmad@uaf.edu.pk , komaljaved666@gmail.com

Pakistan is at the fifth position in cotton production in the world. In cotton trading, it is the third largest exporter of raw cotton. In case of cotton consumption, it is at the fourth number. Cotton is considered as white gold, it performs a splendid role in the economy of Pakistan. The production of the cotton is severely reduced due to *Begomoviruses*. These viruses can cause different diseases in cotton. They produce viral disease in cotton leaves and make leaves curly. The leaf curl virus disease in tomato and some ornamental plants also get the mosaic disease. The disease of the cotton leaf curl virus has reduced the cotton production in the past years. In these days, many techniques are used to remove such problems and to find the remedies against them. Nowadays, an advanced genome editing technique is used called as the CRISPR/Cas9 system. This technique consists of the gRNAs and Cas9 endonuclease. The proposed research was focus on induction of disease resistance against cotton leaf curl virus by using the multiplex CRISPR/Cas system. Agrobacterium mediated transformation was used to deliver the multiplex CRISPR/Cas9 construct into cotton (Coker-312) using medias like MSB and CIM (Callus Induction Media). Moreover, the transgenic callus was used for further downstream analysis i.e. screening of transformation event in cotton via PCR analysis.

0.3.12. ANTIVIRAL PHYTOCHEMICALS IDENTIFICATION, AGAINST NS1 VIRULENCE PROTEIN, TO COPE ZIKA VIRUS INFECTION USING BIOINFORMATICS

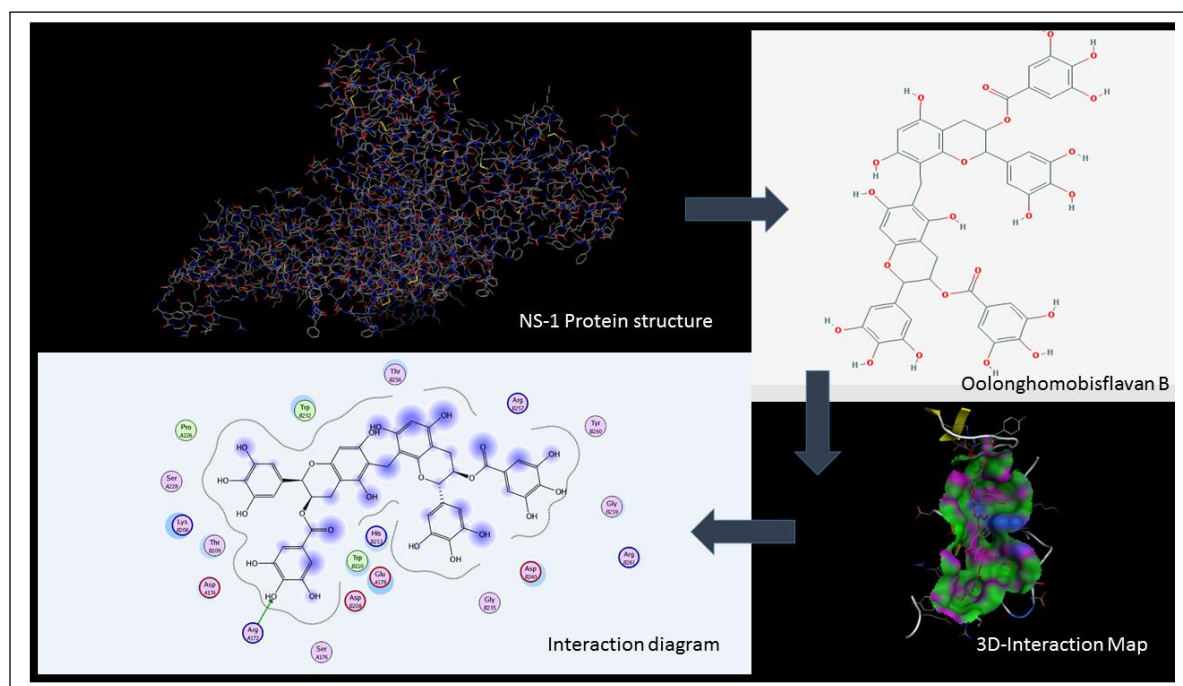
Shakeel Irshad

Centre of Agricultural Biochemistry and Biotechnology (CABB)

University of Agriculture Faisalabad, Pakistan

Corresponding author Email: shakeel_irshad66@yahoo.com

Zika virus (ZIKV) is becoming a serious health issue across the globe. About 0.5-1.3 million cases have been reported since 2015, ZIKV infection, from its mild symptoms to association with serious disorders like microcephaly, macular atrophy and Guillain-Barre syndrome (GBS), is a serious concern. There is no vaccine or drug present against ZIKV that is why we need to find out potential therapeutics to fight against the virus. The purpose of this study is to find out phytochemicals that have antiviral activity against ZIKV NS1 protein, involved in replication and host immune system modulation, with the aid of molecular docking and simulation studies. MOE was used to perform the in silico docking analysis. Results have shown that 7 phytochemicals (Oolonghomobisflavan B, TANNIC ACID, Calceolarioside A, chebulinic acid, 2,3-Dihydroirigenin, Oolonghomobisflavan A, Amentoflavone) shows good binding properties against the NS1 protein of ZIKV. So, from this study we can conclude that Oolonghomobisflavan B may prove to be a potent inhibitor of the NS1 protein of ZIKV.



O.3.13. A COMPUTATIONAL NANOTECHNOLOGY APPROACH FOR SCREENING POTENTIAL THERAPEUTIC NANOPARTICLES AGAINST ALZHEIMER'S DISEASE

Syed Awais Attique¹, Qurat-ul-Ain¹, Syed Asim Atique Shah² and Ghulam Mustafa^{3*}

¹Department of Computer Science, University of Agriculture, Faisalabad-38040, Pakistan

²Department of Computer Science, Air University Islamabad, Pakistan

³Department of Biochemistry, Government College University Faisalabad-38060, Pakistan

Corresponding author Email: drghulammustafa@gcuf.edu.pk

Alzheimer's disease (AD) is a nervous system destructive disease which causes structural, biochemical and/or electrical (neural) abnormalities inside the human brain. Especially gene mutations as well as other factors such as environment, fitness and lifestyle also make contributions to the disease. Latest mode of interest has been growing to become closer to utilize therapeutic perspectives of nanomaterials to cure AD. The in vitro and in vivo approaches are time consuming and they need advanced technologies as well. In this study, we reported nanoinformatics approaches for designing exclusive nanoparticles considering the virulence of nanoparticles and approaches to explore their medicinal potential for treatment of AD. The study was mainly focused on the usage of Lipinski rule of five including rule-of-three to evaluate lead-like and drug-like attributes of nanomaterials. It also enlightened the benefits of computer-aided investigation of nanomaterials for their adsorption, distribution, metabolism and excretion (ADME). The activities of nanoparticles in the body as ligands, their drug likeliness and therapeutic potentials were also explored through using in silico approach. The study provides a novel and clearer insights into the interaction properties of known putative nanoparticles as potential inhibitors of AD.

Keywords: Alzheimer's disease; Nanomaterials; Molecular docking; Lipinski's rule of five; Oral activity; Lead-like attributes

O.3.14. FREQUENCY OF NULL GENOTYPE IN HEALTHY AND LEUKEMIC PATIENTS IN LOCAL SUBJECTS

Naila Rafiq*, Shamaila Rafiq and Tahira Iqbal

Leukemia is a group of diseases that originate in bone marrow. Among four different types of leukemia, Chronic lymphocytic leukaemia (CLL) has several unique features that distinguish it from other cancers. Most CLL tumour cells are inert and arrested in G0/G1 of the cell cycle and there is only a small proliferative compartment; however, the progressive accumulation of malignant cells will ultimately lead to symptomatic disease. GSTM1 is Phase II enzyme that has potential to eliminate xenobiotics that may be carcinogenic. Deletion of the gene may cause susceptibility to leukemia. In Present study the population of CLL patients were checked for GSTM1 deletion polymorphism. The blood collected from 50 patients and 100 healthy volunteers was subjected to PCR and gel electrophoresis. Absence of GSTM1 gene was consider as null genotype. Healthy subjects (38%; 38/100) were found to have lesser null genotype compared to leukemic patients CLL (54%; 27/50).

Keywords: Leukemia, CLL, GSTM1, Null Genotype

O.3.15. RESISTANCE TO CITRUS TRISTEZA VIRUS VIA RNA INTERFERENCE

Muhammad Hassan¹, Saher Naveed¹, Muhammad Waseem Sarwar¹, Ayesha Younus²,
Muhammad Shah Nawaz ul Rehman¹ and Muhammad Mubin^{1*},

Virology Lab, Center of Agricultural Biochemistry and Biotechnology; Department of Plant
Pathology, ²Laser matter interaction and Nano-sciences Lab, Department of Physics,
University of Agriculture, Faisalabad Pakistan

Corresponding author Email: mmubin@uaf.edu.pk

Several fungal, bacterial and viral diseases infect agricultural crops in Pakistan. Citrus is the major fruit crop in Pakistan where Kinnow is the dominant cultivar, accounting for the 80% acreage. Most graft transmissible pathogens of citrus present in Pakistan are transmitted to new plants only by using infected nursery material for propagation. Citrus tristeza virus disease is the most devastating disease of citrus. CTV is an RNA virus and its replication is without any proof reading. Many people have reported different strains of the CTV infecting the same plant. So diversity study is of utmost importance. Previously no data about the citrus tristeza virus from Pakistan at molecular level is available so first there is need to clone and sequence CTV from different regions of Pakistan. After this through RNAi, a strategy of broad-spectrum resistance against this virus is devised. RNA interference is a conserved host defensive mechanism against exogenous nucleic acids including viruses. If we target nucleotide sequence through RNAi (the most conserved region of virus) the resistance will be durable and broad spectrum against all viruses containing that conserved portion.

O.3.16. ENGINEERING PLANTS FOR BEGOMOVIRUS RESISTANCE WITH RNA-GUIDED CAS9 NUCLEASE

Muhammad Salman Mubarik^{1*}, Sultan Habibullah Khan^{1,2}, Zulqurnain Khan³
and Aftab Ahmad^{2,4}.

¹Centre of Agricultural Biochemistry and Biotechnology (CABB), ²Center for Advanced Studies in Agriculture and Food Security (CAS-AFS), ⁴Department of Biochemistry, University of Agriculture Faisalabad (UAF), Pakistan.

³Department of Biotechnology, Muhammad Nawaz Sharif University of Agriculture Multan (MNSUAM), Pakistan.

Corresponding author Email: msmubarik@gmail.com

Begomoviruses infect many economically important crops worldwide. Conventional approaches for resistance management failed to tackle with emerging and rapidly evolving plant viruses. Last decade has witnessed an unprecedented progress in plant genome editing technologies. Among these technologies, the CRISPR-Cas9 system has been successfully applied to establish plant resistance against different plant viruses. In the present study, we demonstrated use of the Cas9-gRNA construct to confer resistance against begomoviruses. We synthesized unique gRNA specific for non-coding region of three most prevalent strains of *Cotton Leaf Curl Virus* (CLCuV) in Pakistan. Using CLCuV infectious clones and Cas9-gRNA construct, transient assays were performed in 3-4 weeks old tobacco (*Nicotiana benthamiana*) plants. The results demonstrated that targeting consensus sequences of CLCuV with Cas9-gRNA complex reduced symptom severity and virus accumulation by 60%-75%. Viral DNA interference was further confirmed by T7E1 DNA double strand break (DSB) assay. The results demonstrated the efficiency of the CRISPR-Cas9 system for the development of virus resistance in plants. Use of this novel strategy can open the possibility to develop resistant plant germplasm against multiple virus infections.

Keywords: *Begomoviruses*, CLCuV, Genome editing, CRISPR/Cas9, Transient assay, Virus suppression.

TRACK 4

INDUSTRIAL/ENZYME BIOTECHNOLOGY, BIOCOMPOSITES AND BIOMATERIALS

TRACK COMMITTEE

- 1. PROF. DR. MUHAMMAD ASGHER (CONVENER)**
- 2. DR. FATMA HUSSAIN**
- 3. DR. NAYLA MUNAWAR**
- 4. DR. ASMA YAQOOB**

KEYNOTE LECTURES

TRACK 4

K-4.1 NATIVE RESOURCES: KEY FOR NATIONAL PROGRESS AND PROSPERITY

Faiz-ul Hassan Nasim

Chemistry Department, The Government Sadiq College Women University Bahawalpur.
Bahawalpur - Pakistan

In this era of knowledge-based economic growth progress of a nation has become associated with the development of technologies that need a variety of raw materials originating from biological sources. Data show that economies of the countries generating raw materials from their own resources are benefitting more from the rapid global technological development. Majority of the newly emerging technologies are bio-based technologies, as living systems have become one of the cheapest sources of the raw materials. Although bio-based technologies have been in use, in crude form, for centuries, the newly developing technologies are heading towards improvement in production and applications and hence performance and sustainability. Time has come to understand renewable chemistry of these technologies to design cost-effective strategies as bio-based products are expected to soon replace the petroleum-based products. Transformed fuel, food and material choices are the targets. Since the global population is expected to increase to 9 billion by 2050, developing economies like Pakistan need to step up their efforts to improve the wealth and health of their human resource. World is looking forward to tap their native biological resources and so is required for the countries like Pakistan. We have vast reservoirs of unexplored biological resources that need to be explored to understand and solve our problems by developing and using the bio-based technologies.

K-4.2 ECO-FRIENDLY LEATHER PROCESSING USING HIGHLY ACTIVE ALKALINE PROTEASES OF BACTERIAL ORIGIN

Hamid Mukhtar

Institute of Industrial Biotechnology, GC University, Lahore-54000, Pakistan

Corresponding author Email: hamidwaseer@yahoo.com

Alkaline protease isolated from *Bacillus subtilis* by submerged fermentation, showing maximum enzyme activity (6.0 U/ml) was employed for dehairing of goatskin. In the lab scale experiments, the process of dehairing was accelerated by the optimization of experimental conditions and by the addition of a penetrant. The complete removal of hairs occurred from the skin pieces after 3 hrs of enzyme treatment at pH 13, 35°C temperature and 3:1 ratio of enzyme and penetrant, respectively, without damaging the hair texture and skin grain pattern. Then the process was scaled up to the industrial level. Soaked goat skins were treated separately, with crude enzyme preparation and concentrated enzyme preparation by dip and paint methods under defined conditions along with the control, lime-sulfide treatment. The best results with the skin processing were obtained when the skin was treated with concentrated enzyme. The quality of wetblue (Area, appearance, plumping, etc) and physical properties of the finally prepared crust leather (Tensile strength and Tear strength) were also improved with the use of concentrated protease. There was remarkable reduction in BOD, COD and TDS of effluent produced as a result of enzymatic treatment.

Keywords: Pollution, effluent, tannery, depilation, enzymatic, protease.

ORAL PRESENTATIONS

TRACK 4

O.4.1. PYROLYSIS OF PLASTIC WASTE INTO FUEL OIL: KINETICS AND PRODUCTS DISTRIBUTION

Jan Nisar*¹, Muhammad Anas Khan¹, Munawar Iqbal², Mazhar Abbas³

¹National Centre of Excellence in Physical Chemistry, University of Peshawar

²Department of Chemistry, University of Lahore, Lahore

³Jhang-Campus, University of Veterinary & Animal Sciences Lahore

This work is focused on the use of a metal impregnated molecular sieve for pyrolysis of polypropylene waste using thermogravimetry and pyrolysis gas chromatography-mass spectrometry. Ozawa Flynn Wall, Tang Wanjun and Coats-Redfern (modified) methods were used for finding out kinetics parameters of the pyrolysis reaction. The lowest activation energy observed confirms the suitability of the catalyst for the pyrolysis reaction. Therefore, the pyrolysis of polypropylene in the presence of same catalyst was performed in an indigenously made furnace. The process involves simple unit operations associated with a liquid fuel production of more than 70% with a wide range of hydrocarbons. Moreover, a few useful aliphatic hydrocarbons were also observed in the gaseous fraction. It was observed that the catalytic degradation of polypropylene performed in an indigenously made furnace in the presence of metal impregnated molecular sieve provides a better selectivity in the product distribution and was demonstrated to be a useful method for the production of potentially valuable hydrocarbons. Finally, the pyrolysis oil composition was compared to the standard parameters of diesel, gasoline and kerosene oil and concluded that pyrolysis oil from polystyrene waste have potential application replacing fuel oil.

O.4.2. BIOCHEMICAL CHARACTERIZATION OF XYLANASE BY LOCALLY ISOLATED ASPERGILLUS SPECIES

Imran Ali, Attiq U Zaman, Rehana Asghar and Raja Tahir Mahmood

Department of Biotechnology, Mirpur University of Science and Technology (MUST),

Mirpur AJK

Corresponding author Email: aliimran@must.edu.pk

Hemicellulose is major constituent of plant biomass and xylanase hydrolyzes β -1-4, glycosidic bonds in the structure of xylan. The current study was aimed to optimize the cultural conditions for the maximum production of xylanase by aspergillus species. Enzyme was purified by ammonium sulfate precipitation and gel filtration chromatography. Purified enzyme was used for the characterization of temperature, pH and kinetic parameters. Maximum activity of xylanase was observed after 96 hours of incubation, at 40⁰C and 7 pH (2.121 IU/mL/min). Among various carbon source, wheat bran showed maximum xylanase activity (2.155 IU/mL/min) followed by maize bran (2.001 IU/mL/min) and xylan (1.972 IU/mL/min), while saw dust showed least activity (0.124 IU/mL/min). Trypton as a nitrogen source supported higher production of xylanase 1.816 IU/mL/min compared to beef extract (1.749 IU/mL/min). In case of carbon source (wheat bran), maximum production of xylanase was observed at pH 6 (1.977 IU/mL/min), significant xylanase production was observed at pH 5 (1.888 IU/mL/min) and at pH 4 also (1.841 IU/mL/min). After addition of nitrogen source (trypton) maximum xylanase production was obtained at pH 5 (1.788 IU/mL/min), significant activity at pH 6 (1.712 IU/mL/min) and at pH 7 also (1.703 IU/mL/min). The presence of 20 % ammonium sulphate gave maximum precipitation with activity 1.171 IU/mL/min and activity increased upto 2.224 IU/mL/min after gel filtration chromatography. Optimum pH and temperature of enzyme were 5 and 50 ⁰C respectively. The activity of xylanase was inhibited by ZnSO₄ and FeSO₄ while it was stimulated in the presence CuSO₄ and CaCl₂. The activity of enzyme was enhance in the presence of organic solvents like glycerol and methanol while decreased ethanol and isopropanol. The activity was slightly decreased by 0.25 % SDS. Xylanase activity was decreased with the increasing concentration of inhibitor (EDTA). The V_{max} and K_m of xylanase were calculated as 4409.17 μ M/mL /min and 1.982 mM respectively.

Keywords: Xylanase, hemicellulose, xylan, wheat bran, ammonium sulfate

O.4.3. SIMULTANEOUS *PRETREATMENT* AND BIOHYDROGEN PRODUCTION FROM WHEAT STRAW BY NEWLY ISOLATED LIGNINOLYTIC BACILLUS SP. STRAINS WITH TWO-STAGE BATCH FERMENTATION SYSTEM

Tawaf Ali Shah^{ab}, Shehbaz Ali^{ab}, Romana Tabassum.*^{ab}

^aNational Institute for Biotechnology and Genetic Engineering
(NIBGE), Faisalabad, Pakistan

^bPakistan Institute of Engineering and Applied Science (PIEAS), Islamabad, Pakistan

Corresponding author: Tawaf Ali Shah

Current address: * Corresponding author at: National Institute for Biotechnology and Genetic Engineering (NIBGE), Jhang road 3800, Faisalabad, Pakistan

Corresponding author Email: tawafbiotech@yahoo.com

Biodegradation of agribiomass especially wheat straw to biohydrogen and biomethane is an encouraging approach to the current waste management problem. To do so, the biomass must first be pretreated to break down lignin thereby increasing accessibility of the substrate to fermentative organisms. In the current study, out of 20 isolates from the granular sludge of full-scale anaerobic digester, four ligninolytic *Bacillus* sp. strains were selected based on their lignin and Azure B degradation. Further among the four isolates, *Brevibacillus agri* AN-3 exhibited the highest of 88.4 and 78.1% decrease in COD of lignin and Azure B respectively. These strains were also found to secrete optimum yields of lignin peroxidase (LiP) at pH 3, laccase (Lac) at pH 5, xylanase and cellulase enzymes at pH 7. The strains demonstrated maximum activity of Lip and Lac at 50 °C and xylanase and cellulase at 60 °C after 72 h growth. Among the four strains, *Brevibacillus agri* AN-3 showed hydrogen (H₂) yield of 1.34 and 2.9 mol-H₂/mol from xylose and cellulose respectively. In two-phase wheat straw batch fermentation, *Brevibacillus agri* AN-3 produced 88.3 and 283.7 mL/gVS cumulative H₂ and methane (CH₄) respectively. Biotreatment with ligninolytic *Bacillus* sp. strains perceived that 261.4 % more methane yield could be obtained from the wheat straw than using the untreated wheat straw in batch fermentation. This is the first study establishing not only the hydrogen potential of ligninolytic *Bacillus* sp. strains but also indicates a vital role of these species in developing standard inoculum and a biocatalyst for processing agribiomass.

Keywords: anaerobic ligninolytic; fermentation; *Bacillus* sp. strains; lignocellulosic waste biomass

O.4.4. PRODUCTION, PURIFICATION AND CHARACTERIZATION OF EXOGLUCANASE FROM LOCALLY ISOLATED *ASPERGILLUS FLAVUS*

Noreen Rafiq^{1*}, Muhammad Altaf Hussain¹, Qurat ul Ain¹

¹ Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250, (AJK), Pakistan

Lignocellulosic biomass is considered as plentiful organic raw material in the world. Due to the depletion of oil reserves and shortage of food the consumption of cellulosic biomass is considered to be a subject of worldwide interest. The current study was aimed to optimize the cultural conditions for maximum production of exoglucanase by *Aspergillus flavus*. Wheat straw was used as a substrate. The parameters were optimized like incubation period, pH, temperature, substrate level, moisture level, inoculum size, addition of various readily available carbon and nitrogen source for higher yield of exoglucanase enzyme. By gel filtration chromatography and ammonium sulphate precipitation, enzyme was partially purified. The enzyme that is purified has been characterized for optimum pH and temperature. Effect of different metals ions, effect of solvents, effect of inhibitors, and effect of non-ionic surfactants on enzyme activity was also monitored. The maximum activity of exoglucanase was observed at 72 hour of incubation, 40°C, 7 pH, 5mL, inoculum size, 60 % moisture level, 8g substrate level. Different combinations of nitrogen and carbon sources had no effect on the production of exoglucanase. The maximum exoglucanase activity was conferred by adding 70% ammonium sulphate. Exoglucanase activity was increased by Mn^{2+} , Cu^{+2} , Co^{+2} , Ca^{+2} , Mg^{+2} while Zn^{+2} slightly decreased exoglucanase activity. Hg^{+2} and Fe^{3+} was inhibited the exoglucanase activity.

**O.4.5. COMPARISON OF BIODEGRADATION POTENTIAL OF SELECTED
DISPERSE TEXTILE DYES BY DAEDALEA DICKINSII IEBL-02 AND
PIPTOPORUS BETULINUS IEBL-03 AND STUDY OF LIGNINOLYTIC ENZYMES**

Raja Tahir Mahmood¹, Sufia Tazeen¹, Muhammad Javaid Asad², and Imran Ali¹

¹ Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 AJK, Pakistan

² Department of Biochemistry, PMAS-Arid Agriculture University Rawalpindi, Pakistan

Corresponding author Email: raja.tahir@must.edu.pk

Current study was designed to investigate the ability of *Daedalea dickinsii* IEBL-02 and *Piptoporus betulinus* IEBL-03 to decolorized disperse textile dyes. Biodegradation of disperse Yellow SRLP, and disperse Red S3B was monitored along with secretion of lignolytic enzymes. The decolorization process was observed for 10 consecutive days with the analysis of process on each day. The results showed that *Daedalea dickinsii* IEBL-02 (70-75 %) has the more potential of biodegradation of above mentioned disperse dyes while *Piptoporus betulinus* IEBL-03 (47-53 %) has the least. The biodegradation process of dyes was optimized by Response Surface Methodology with *D. Dickinsii* IEBL-2 and more than 85 % biodegradation was achieved. The study of ligninolytic enzymes i.e. lignin peroxidase, manganese peroxidase and laccase showed that *D. dickinsii* IEBL-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 °C and pH 5.8 with good affinity towards their substrates as indicated by values of Km and Vmax. This study will bring the attention of other scientist to use brown rot fungi for the treatment of wastewater.

Keywords: Response Surface Methodology, Ligninolytic enzymes, Wastewater, textile dyes

O.4.6. BIOCATALYTIC DEGRADATION OF VARIOUS DYES BY A NOVEL PEROXIDASE FROM SOYBEAN

U.Kalsoom^{1*}, H. N. Bhatti², S. Salman Ashraf³

¹Chemistry Department, GC Women University Faisalabad

²Chemistry Department, University of Agriculture, Faisalabad, PAKISTAN

³Chemistry Department, P. O. Box 17551, UAE University, Al-Ain, UAE

Corresponding author Email: kalsoom5800@yahoo.com

Peroxidases are emerging as an important class of enzymes that can be used for the efficient degradation of textile and non textile dyes. The present study revealed the potential of soybean peroxidase towards degradation of series of dyes such as Brilliant Yellow, Methyl Blue, Naphthol Blue Black, Congo Red, Tartrazine, Rhodamine B, Toluidine Blue, Orange G, Naphthol Yellow, Trypan Blue. Recalcitrant dyes could also be mineralized by soybean peroxidase in the presence of redox mediator. It was also observed that Soybean Peroxidase could be successfully immobilized in Polyacrylamide gel and used up to 6 times without significant loss of activity. In order to confirm the dye degradation HPLC-DAD and LC/MS studies were carried out to and analyzed the intermediate metabolites. The results showed that Soybean peroxidase causes Trypan Blue degradation via symmetrical azo cleavage and subsequent radical-initiated ring opening of the metabolites. Hence, plant peroxidases are easily available, inexpensive and ecofriendly biocatalysts for the treatment of waste waters containing a wide spectrum of textile and non-textile synthetic dyes.

Keywords: Dyes; Peroxidases; Degradation; Wastewaters

O.4.7. TOPOPYRONE C DERIVATIVES, CREDIBLE TOPOISOMERASE-1 ENZYME INHIBITORS

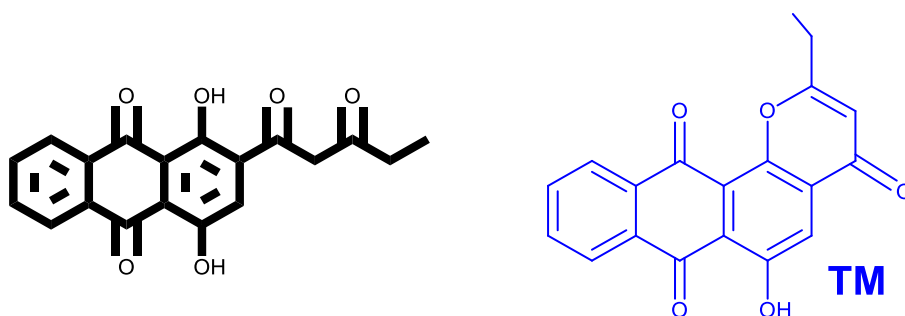
Shahid Aziz

Department of Chemistry, Mirpur University of Science & Technology (MUST)

Mirpur, AJK.

Corresponding author Email: drshahid.chem@must.edu.pk

Topopyrones are known for their anti-cancer activity. Topopyrone C is the main inhibitor of topoisomerase-1 enzyme. Different routes for the synthesis of topopyrone C derivative are reported. The 1, 4-dihydroxyanthraquinone was converted to target compound (TM) using five step sequences, while only three steps were required to access the said compound, starting from the same anthraquinone. A new one-pot alkylation served as the crucial operation for this new synthesis. Two lengthy steps are eliminated which not only shorten the reaction but improve yield too. Spectral techniques were used to confirm the structures of synthesized compounds. The compound exhibited strong scavenging effect on DPPH, NO and SO radicals. The free radical scavenging effect of the compound was found comparable to that of reference antioxidants BHA and ascorbic acid. Cytotoxic activity of compound was investigated against three different cancer cell lines, Hep-2, RD and L-20B and a non cancerous cell line VERO. No cytotoxic effect on VERO cell line was found in the tested experiments.



Key words: Topopyrones, efficient synthesis, biological activities.

O.4.8. ENHANCED ETHANOL PRODUCTION FROM LIGNOCELLULOSIC WASTES BY AN INDIGENOUSLY ISOLATED MIXED CULTURE OF *SACCHAROMYCES* AND *CANDIDA* USING BOX BEHNKEN DESIGN

Shagufta Kamal^{1*} · Ismat Bibi² · Abdullah Ijaz Hussain³ · Naheed Akhter⁴ · Razia Noreen¹ ·

Saima Rehman³ · Tanvir Ahmed⁵ · Sumbel Maqsood¹ · Nadeem Abbas Faisal³

¹Department of Biochemistry, Government College University, Faisalabad 38000, Pakistan

²Department of Chemistry, Islamia University, Bahawalpur 63100, Pakistan

³Department of Chemistry, Government College University, Faisalabad 38000, Pakistan

⁴Department of Allied Health Professionals, Government College University, Faisalabad 38000, Pakistan

⁵Department of Statistics, Government College University, Faisalabad 38000, Pakistan

Corresponding author Email: shaguftakamal@gcuf.edu.pk

Corn stover is readily available due to the proximity of corn grain and hence can be the prime source of bioethanol. High degree of complexity due to mixed composition of non-homogeneous fiber is the main hurdle in its use. In the present study, corn stover has been used for bioethanol production using statistically optimized simultaneous scarification and fermentation by locally isolated and identified strain of *Aspergillus niger* *Saccharomyces cerevisiae* (IBL-01) and *Candida cantarellii* (IBL-01). Regression analysis and Box Behnken design (3 significant factors at 3 levels) were used to analyze the effect of the process parameters i.e. temperature 35°C to 41 °C, pH 4.5-7.5 and inoculum size 1mL to 5mL on the final yield of ethanol. NaOH pre-treatment increased cellulose composition by 19.53% accompanied by 13.98% decrease of lignin contents. HPLC analysis revealed that concentration of glucose 2.45 mg/mL, mannose 1.67 mg/mL, fructose 1.008 mg/mL after saccharification with *Aspergillus niger* whereas the activities of exoglucanase, endoglucanase, and β -glucosidase were 41.3 ± 1.31 , 53.5 ± 1.24 and 46.8 ± 1.43 U/mL respectively. Every step in SSF decreased the degree of polymerization and crystallinity of corn stover which was confirmed by SEM analysis. The results showed that maximum ethanol concentration obtained after SSF was $18.50\text{mg}/100\text{mL} \pm 1.00$ after 6h at 35 °C with 5mL inoculum and pH 6. The results suggested that BBD using indigenously isolated microbe is a useful tool for optimum yield of bioethanol from agro-industrial wastes.

Keywords: Corn stover, Bioethanol, Regression analysis, Box Behnken design

O.4.9. PURIFICATION AND EVALUATION OF ANGIOTENSIN I- CONVERTING ENZYME INHIBITORS RELEASED BY ENZYMATIC HYDROLYSIS OF GOAT MILK CASEIN

Iqra Aslam^{a*}, Sadia Javed^a, Munazzah Meraj^b, Farkhanda Arshad^a, Muhammad Numan^a

^aDepartment of Biochemistry, Govt. College University Faisalabad, Pakistan

^bDepartment of Biochemistry, Peoples University of Medical and Health Sciences for
Women, Nawabshah, Pakistan

Corresponding author Email: iqraaslam055@gmail.com

This project was planned to study the angiotensin I-converting enzyme inhibitory (ACE- I) activities of peptides generated from goat milk casein to employ for therapeutic purposes in hypertension. Casein from goat milk was hydrolyzed by various proteolytic enzymes such as pepsin, trypsin and combination of pepsin and trypsin. The degree of hydrolysis and ACE-I activity was measured from casein. The goat milk casein hydrolysate at 40 h showed highest ACE inhibition ratio (92.4%). So, this hydrolysate from goat milk was selected for further purification. This hydrolysate was fractioned into 6 fractions (F-1 to F-6) by ion exchange chromatography and fraction of highest ACE-I activity was purified by gel chromatography. The final purification of the fractions F-3a to F-3h, obtained from gel filtration chromatography, was done by using HPLC. The F-3d fraction showed maximum inhibitory activity among all fractions. Finally, F-3d fraction was run on electrophoresis and a single band was obtained. These results showed F-3d as single peptide and the amino acid sequence of this peptide was found to be Ala-Tyr-Phe- Tyr, Pro-Tyr-Tyr. Conclusively, the goat casein hydrolysate can inhibit the ACE enzyme and could be promising at decreasing blood pressure. Moreover, this study provides helpful insights determining the potential application of goat milk as a component of antihypertensive functional foods.

Keywords: Angiotensin I-converting enzyme; Hypertension; Inhibition; Casein

O.4.10. ISOLATION AND CHARACTERIZATION OF THIOPHENIC COMPOUND DESULFURIZING BIOCATALYSTS FROM OIL CONTAMINATED SITES

Sana Parveen, Nasrin Akhtar*, Kalsoom Akhtar and Muhammad A. Ghauri

Industrial Biotechnology Division, National Institute for Biotechnology and Genetic
Engineering, P. O. Box 577, Jhang Road, Faisalabad, Pakistan.

Corresponding author Email: nasrin_379@yahoo.com, nasrin@nibge.org

Combustion of sulfur loaded fossil fuels releases a great amount of sulfur oxides into atmosphere that causes environmental pollution and serious health problems. To reduce the sulfur levels, chemical process of hydrodesulfurization (HDS) is being used in oil refineries; however, this process demands high operating conditions and is inefficient in removing sulfur from recalcitrant thiophenic compounds such as dibenzothiophene (DBT). Biodesulfurization is a potential complementary technology to HDS process as it operates under mild conditions and removes thiophenic compounds that are resistant to HDS. The aim of current study was to isolate and characterize the potential DBT desulfurizing biocatalysts from oil contaminated sites. For this purpose a total of 51 soil, sand, water and sludge samples were collected from the oil contaminated areas of OGDCL Rajian, Chakwal. The samples were enriched in selective media containing DBT as a sole source of thiophenic sulfur and about 40 pure bacterial isolates were obtained from these mixed cultures. Among 40 pure cultures, seven isolates (W3SN5, W3SN4, W3SL1, W3SN2, W3SN1, W3S2-P9 and W3S5) were able to convert DBT into 2-hydroxybiphenyl (the end product of DBT desulfurization 4S pathway) as determined through Gibb's assay and HPLC analysis. The isolated DBT metabolizing biocatalysts were characterized through 16S rRNA gene sequencing followed by nucleotide BLAST homology search and phylogenetic analysis (MEGA 5). Six of the isolated biocatalysts showed 99-100% 16S rRNA gene sequence homology to different species of the genus *Gordonia* while the isolate W3SN5 showed 100% 16S rRNA gene sequence homology to *Kocuria polaris*. Phylogenetically the isolates W3S2-P9, W3S4, W3SN1, W3SN2 and W3SL1 formed a separate group with different species of genus *Gordonia* including *G. terrae*, *G. hongkongensis* and *G. didemni*. However, isolates W3SN4 and W3S5 formed a separate group with *G. alkanivorans*, *G. amicalis*, and *G. rubripertincta*. The isolate W3SN5 formed a completely separate clade with *Kocuria Polar*is. The 16S rRNA gene sequences of these isolates were submitted to GenBank under accession numbers MH569666-MH569672. The capabilities of the isolated biocatalysts to survive and efficiently desulfurize the thiophenic sulfur containing compounds like DBT make them potential candidate for biorefining of oil in terms of sulfur removal.

**O.4.11. COMPARISON OF BIODEGRADATION POTENTIAL OF SELECTED
DISPERSE TEXTILE DYES BY DAEDALEA DICKINSII IEBL-02 AND
PIPTOPORUS BETULINUS IEBL-03 AND STUDY OF LIGNINOLYTIC ENZYMES**

Raja Tahir Mahmood¹, Sufia Tazeen¹, Muhammad Javaid Asad², and Imran Ali¹

¹ Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 AJK, Pakistan

² Department of Biochemistry, PMAS-Arid Agriculture University Rawalpindi, Pakistan

Corresponding author Email: raja.tahir@must.edu.pk

Current study was designed to investigate the ability of *Daedalea dickinsii* IEBL-02 and *Piptoporus betulinus* IEBL-03 to decolorized disperse textile dyes. Biodegradation of disperse Yellow SRLP, and disperse Red S3B was monitored along with secretion of lignolytic enzymes. The decolorization process was observed for 10 consecutive days with the analysis of process on each day. The results showed that *Daedalea dickinsii* IEBL-02 (70-75 %) has the more potential of biodegradation of above mentioned disperse dyes while *Piptoporus betulinus* IEBL-03 (47-53 %) has the least. The biodegradation process of dyes was optimized by Response Surface Methodology with *D. Dickinsii* IEBL-2 and more than 85 % biodegradation was achieved. The study of ligninolytic enzymes i.e. lignin peroxidase, manganese peroxidase and laccase showed that *D. dickinsii* IEBL-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 °C and pH 5.8 with good affinity towards their substrates as indicated by values of Km and Vmax. This study will bring the attention of other scientist to use brown rot fungi for the treatment of wastewater.

Keywords: Response Surface Methodology, Ligninolytic enzymes, Wastewater, textile dyes

O.4.12. SYNTHESIS OF WOOD PLASTIC BIO COMPOSITES INDUCED WITH BIO-WASTE MATERIAL

Nadia Akram,^{*a} Tanzeel Munawar^a

^A Department of Chemistry, Government College University, Faisalabad-38030 Pakistan

Corresponding author Email: nadiaakram@gcuf.edu.pk

PVC is the third abundantly used thermoplastic polymer. It is widely used in many valuable products. The bio-waste is one of the major sources of raw materials available in various forms. The plant generated bio-waste is a cheap and abundant source of filler to produce the bio-composites. Wood Plastic Bio Composites (wpbcs) was made of wood fiber and thermoplastic materials including Poly Vinyl Chloride (PVC). In the present study the bio-waste consisting of rice husk was used for the synthesis of biocomposites containing PVC as matrix. The different series of PVC films was synthesized by using various molecular weight of PVC. The PVC films of different thickness were prepared. The biocomposites was prepared using rice husk, which was varied in weight percentage (wt. %). The quantity of filler was optimized in order to obtain the maximum output. The synthesized samples were subjected to the structural characterization by using IR, TGA and DMA. The results of TGA revealed that in the PVC/rice husk composite of lower molecular weight (48,000 g/mol) the thermal stability was increased up to 10 %. While PVC/rice husk composites of higher molecular weight (62,000 g/mol) the thermal stability was decreased up to 10 % as compared pure PVC films. The results of DMA revealed that in the PVC/rice husk composite composite of lower molecular weight (48,000 g/mol) the storage modulus was increased up to 25%. While composite of higher molecular weight (62,000 g/mol) the storage modulus was decreased up to 20 % as compared with pure PVC films.

Keywords: Biowaste, Poly vinyl Chloride, FTIR, TGA, Mechanical properties

O.4.13. SORPTION OF CRYSTAL VIOLET FROM AQUEOUS MEDIA BY BIOCOMPOSITES

Aqsa Tahir, Haq Nawaz Bhatti* and Amina Khan

Department of Chemistry, University of Agriculture, Faisalabad-Pakistan

Corresponding author Email: hnbhatti2005@yahoo.com

Batch study was performed to investigate the potential of saw dust (SD) and its biocomposites for the removal of crystal violet from contaminated aqueous media. Different composites with polyaniline (PAN/SD), polypyrrole (Ppy/SD), sodium alginate (Na-Alginate/SD) were synthesized and used as efficient adsorbent for removal of crystal violet from water. The effect of different process parameters such as contact time, temperature, adsorbent dose, pH and initial dye concentration has been evaluated. The results indicated that maximum adsorption capacity was observed at pH 7 for SD and Na-Alginate/SD while at 6, 8 for Ppy/SD and PAN/SD respectively. Temkin, Freundlich, Langmuir, Harkins Jura and Dubinin-Radushkevich isotherm models were applied to experimental equilibrium data of dye. Different kinetics models like pseudo first and second order were applied. It was observed that pseudo second order was best fitted to experimental data. Thermodynamics parameters of adsorption like Free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) were also calculated, which proved the exothermic and spontaneous nature of reaction. The FTIR results showed the presence of different groups like amino, hydroxyl and carboxylic acids on the surface of biosorbents. The results indicated that saw dust and its composites could be used for the remediation of crystal violet containing wastewater.

Keywords: Crystal violet; Removal; Kinetics; Saw dust; Thermodynamics

O.4.14. MUTAGENESIS OF BACILLUS SUBTILIS AND THE PROCESS OPTIMIZATION FOR PRODUCTION OF BIOSURFACTANT

Mariam Afzal, Muhammad Asgher, *Nimrah Khalid, Sarmad Ahmad Qamar
Industrial Biotechnology Lab., Department of Biochemistry, University of Agriculture,
Faisalabad, Faisalabad-38000, Pakistan

Corresponding author Email: nimrahkhalid090@gmail.com

Biosurfactants are biologically active compounds produced by different microbial strains having hydrophilic and hydrophobic compounds. They are best known for its ability to reduce surface tension and help two immiscible solvent to dissolve properly by reducing their surface tension. They are best competitor of synthetic surfactant in term of safety, low toxicity, high biodegradability and eco-friendly nature. It is used in many industries as emulsifiers, conditioners, cosmetics and food industries. In this study, *Bacillus subtilis* was used for the production of high quality biosurfactant with low production cost and maximum yield. To enhance the yield of biosurfactant, mutagenesis of *Bacillus subtilis* was done using ethidium bromide at different concentration. Best producing mutant strain (42.46 ul/mL) was selected with high yield of biosurfactant. Optimization was done using response surface methodology (RSM) under central compost design (CCD). Maximum biosurfactant production was 49.65 ul/mL at pH 7, inoculum size 3 mL, incubation time 72 hours and 30°C temperature. Presence of biosurfactant was conformed by using different test such as emulsification index and oil displacement. In this study waste automobile oil was used for the production good quality biosurfactant. This can be the best method for the production of useful and environment friendly compounds and help to overcome environmental pollution.

TRACK 5

CHEMICAL AND PLANT SCIENCES

TRACK COMMITTEE

- 1. PROF. DR. HAQ NAWAZ BHATTI (CONVENER)**
- 2. DR. SHAUKAT ALI**
- 3. DR. MUHAMMAD ZAHID**
- 4. DR. BUSHRA SULTANA**

KEYNOTE LECTURES

TRACK 5

K-5.1 SYNTHESIS AND CHARACTERIZATION OF MAGNETIC POLY (ACRYLIC ACID) HYDROGEL FABRICATED WITH COBALT NANOPARTICLES FOR ADSORPTION AND CATALYTIC APPLICATIONS

Tariq Mahmood Ansari*, Muhammad Ajmal and Sadia Saeed

Institute of Chemical Sciences, Bahauddin Zakariya University, Multan 60800, Pakistan

Corresponding author Email: tariqansari@bzu.edu.pk

In this study, magnetic poly (acrylic acid) hydrogel was prepared by free radical polymerization reaction. The hydrogel was then fabricated with Cobalt nanoparticles by loading Co (II) ions from aqueous solution and their subsequent reduction with NaBH₄. Fabrication of Co nanoparticles in hydrogel resulted in magnetic characteristics and also increased the adsorption capacity. The hydrogel was characterized by Fourier Transform Infrared Spectroscopy (FT-IR) and Transmission Electron Spectrometry (TEM). The hydrogel was used as an adsorbent for removal of methylene blue from aqueous solution. 98% removal of methylene blue was achieved. Three adsorption isotherms were applied to the adsorption data. Results showed that Fruendlich isotherm model was followed with R^2 (0.95). The application of pseudo first order and pseudo second order kinetics on the adsorption data revealed that pseudo second order kinetics was followed in this case. The hydrogel was also used for catalytic reduction of a toxic pollutant i.e. 4-Nitrophenol. The reduction of 4- Nitrophenol followed pseudo first order kinetics. Activation energy and k_{app} were calculated as 13.35 kJ/mole and 0.24 min⁻¹ respectively. Recycling of the magnetic poly (acrylic acid) hydrogel fabricated with Cobalt nanoparticles was carried out for four consecutive cycles and no significant loss in catalytic activity was observed.

K-5.2. POTENTIAL OF SOIL EXTRACTED HUMIC ACIDS ON MINERAL NUTRIENTS UPTAKE

Tajnees Pirzada

The investigation was carried out for evaluation of binding ability of humic acid (HA) with the nutrients metal ions. HAs were extracted from local agriculture lands by International Humic Substances Society (IHSS) method. HAs were characterized by UV-Visible spectroscopy to analyse the ability of isolated HAs binding with K(I), Zn (II) and Fe (III) ions. Then the complexes of isolated humic acids and Aldrich Standard HA with potassium, zinc and ferric ions were prepared. pH was optimized for the maximum complexes formation was found 8, 7 and 6 respectively. Concentration of nutrient ions were detected in isolated HAs as well as its complexes by Atomic Absorption Spectrophotometer (AAS). Moisture and electrical conductivity (EC) indicated the better water holding ability of soil and texture indicated silt-loam type. Alkaline pH values proved good fertile soil, beneficial for crop. The optical parameter E_4/E_6 ratio indicates molecular condensation of extracted HAs possessing high molecular condensation, hydrophilic and aromatic nature. Finally the presence of higher concentration of metal ions expressed the chelating capability of HAs.

K.5.3. PHYTOSANITARY RISK MANAGEMENT PROGRAM IN PAKISTAN – NEXT GENERATION PEST MANAGEMENT AND AFLATOXINS DETERMINATION IN HORTICULTURAL COMMODITIES

Sabyan Faris Honey*

Centre for Agriculture and Bioscience International (CABI), Regional Centre for Central and West Asia, Rawalpindi, Pakistan.

Corresponding author Email: s.honey@cabi.org

Food security is one of the main issues of growing population in current century. Horticulture which is an important subsector of agriculture in Pakistan deals with majority of dietary ingredients necessary for human health. Recently high infestation of certain pests and contamination of horticultural produce with aflatoxins – *Aspergillus flavus* resulted in reduced production and less exports from country. CAB International through its project “Phytosanitary Risk Management Program in Pakistan (PRMP)” initiated its interventions to cope with these issues. The objective of the program was to strengthen the capacity of national agricultural system to deploy next generation pest management techniques with special reference to biological control of pests of concerns and to determine the prevalence of aflatoxins along supply chain of selected horticultural commodities. PRMP executed biological control program in Sindh, Balochistan and Gilgit Baltistan. The technologies were developed in Biological Control Laboratories established at all project sites in collaboration with local stakeholders (Department of Agriculture Research & Extension in each province). Project enhanced capacity of farmers, traders and national officials to support and implement PRMP interventions. Deployed technologies resulted in complete eradication of papaya mealybug from Karachi and 1st reports of establishment of biocontrol agents of giant mealybug at farmer’s field in Skardu region. While efforts for fruit fly in Gilgit region and for apple codling moth and spider mites in Balochistan are being continued. Besides these, comprehensive surveys for selected horticultural commodities (Chili and Maize) were conducted in Punjab and Sindh, and samples were subjected to NGS tool aid screening for aflatoxins determination. The NGS study was broadened to incorporate metabarcoding (using ITS and 16S) to determine the microbial biodiversity of the soil samples and screening for the presence of toxigenic strains. Moreover, whole genome sequencing (WGS) with low coverage techniques was employed to screen the isolates for molecular differences in the ribosomal RNA gene cluster and mitochondrial genome. Localities with aflatoxins contamination were determined and categorized based on genomic analysis. Successful implementation of PRMP will result in enhanced capacity of national agricultural system to implement biological control programs for target pests and to design further studies on aflatoxins mitigation in country.

Keywords: Aflatoxins, Biological Control, NGS Tools, Whole Genome Sequencing, PRMP.

ORAL PRESENTATIONS

TRACK 5

O.5.1. GUAIACOL PEROXIDASE EXPRESSION UNDER SALINITY STRESS IN TWO ECOTYPES OF *VETIVERIA ZIZANIOIDE*

Ayesha Sumreen¹, Faiz-ul Hassan Nasim^{2*}, Samina Ejaz³, Hafiz Shahbaz Mahmood⁵, Asma Yaqoob⁶, Muhammad Shafiq Chaudhry⁴ and Muhammad Ashraf¹

Department of Chemistry¹, Department of Biochemistry and Biotechnology³, Department of Life Sciences⁴, The Islamia University of Bahawalpur, Bahawalpur-63000, Pakistan.²Department of Chemistry, Government Sadiq College Women University, Bahawalpur, 63100, Pakistan. ⁵Department of Entomology, University of Agriculture, Faisalabad 38000. Pakistan. ⁶Department of Biochemistry, University of Agriculture, Faisalabad., 38000. Pakistan

*Corresponding author Telephone: (92-62) 9255473/03216807761, 03336386356

Corresponding author Email: faiz.nasim@hotmail.com

On exposure to various abiotic stresses plants activate a complex set of defense activities that ultimately leads to morphological, physiological, biochemical and metabolic changes. Main focus of this study was to document variations in the expression profile of an antioxidant enzyme, Guaiacol peroxidase (GPX), in two ecotypes of a Cholistan desert grass, *Vetiveria zizanioide*, on exposure to various levels of salinity stress (0 to 350 mM NaCl). Plants were grown hydroponically. Various morphological, physico-chemical and biochemical features were examined and the data was analyzed statistically. Plant proteins extracted in phosphate buffer (pH 7) were quantified using Bradford assay. The crude extracts were subjected to quantitative assays to document activity of GPX. GPX isoform expression profile was established using enzyme specific native gel electrophoresis assays. Gel images were subjected to densitometric analysis that helped to identify 8 isoforms of GPX in both ecotypes. Among eight isoforms GPX-1, 2, 6 and 8 appeared to be involved in stress management and tolerance. Interestingly, in almost all tissues the pattern of isoforms expression was associated with the strength of the applied salt stress. The information thus obtained provides some insight but also demands further investigation to explore these responses at molecular levels.

Keywords: Vetiver grass (*Vetiveria zizanioide*), Abiotic stress, Antioxidant enzymes/isozymes, Guaiacol peroxidase (GPX), Zymography.

**O.5.2. BIOCHEMICAL DIAGNOSIS OF CITRUS HUANGLONGBING AND
ELIMINATION OF *CANDIDATUS LIBERIBACTER ASIATICUS*
BY USING PENICILLIN**

Muhammad Sarwar Yaqub¹ and Rozina Aslam²

¹Department of Horticultural Sciences; ²Department of Chemistry,
The Islamia University of Bahawalpur, Pakistan

Corresponding author Email: hlb92@yahoo.com

Huanglongbing (HLB), also known as citrus greening, is one of the most devastating diseases of citrus worldwide menacing the survival of Pakistan and world's citrus industry. HLB is caused by different species of *Candidatus Liberibacter* bacterium: *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter americanus* and *Candidatus Liberibacter africanus*. The bacterium is transmitted from one plant to other by psyllid vector and through budding/grafting of infected plant material. As potential control strategies for citrus HLB, the effectiveness of different doses of penicillin including 25ppm, 50ppm, 75ppm and 100ppm in solution form were evaluated on HLB infected scions grafted on rough lemon. Infected grafts were immersed in penicillin solutions for 2, 4, 6 and 8 hours. Biochemical analysis for the elimination of bacterial pathogen from treated sprouts was performed using iodo starch test. Survival of disease free scion after the treatment with antibiotic is landmark for HLB management. Our results revealed that scions immersed for 8 hours in 25ppm solution of penicillin provided the highest efficiency in graft survival, sprouting and suppressing the HLB. This may provide a useful tool for the management of citrus HLB.

Keywords: Asian citrus psyllid, Citrus greening disease, Gram negative, Iodo starch test

O.5.3. EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI TO REDUCE SALINITY STRESS IN PEA PLANT

Salma Akhter and Syeda Asma Bano

Department of Microbiology, University of Haripur

Present research project was focused on testing the salinity stress conditions on *Pisum sativum* plants as a result of inoculation of Arbuscular Mycorrhizal fungi. A green house pot experiment was performed where three salt treatments were used. There were two experimental sets; one was provided with salt concentrations and inoculum of Arbuscular mycorrhizal fungi and other was non-inoculated and provided with different salt concentrations. Three different concentration of sodium chloride T1, T2, and T3 (100mM, 200mM and 300mM) were prepared. Autoclaved soil was used as a medium to grow the *Pisum sativum*. Different salt treatments were applied after two weeks of planting seedlings. Growth parameters eg. Root and shoot lengths, and root, shoot fresh weight were analyzed among different treatments and also compared with the controls. The chlorophyll and carotenoid content were also analyzed. Percentage root colonization of the inoculated samples was also tested. The samples of set 1 showed increase in fungal colonization as it was inoculated with AM fungi. The AM inoculated samples T1, T2 and T3 showed a trend of decrease in fungal colonization, but this decrease in colonization was not significant. The Chlorophyll and carotenoid contents were increased in AM inoculated salt treated plants as compared to the control treatments. The lower Na⁺ accumulation in the leaves was observed in the AM inoculated salt treated plants as compared to the un-inoculated salt treated plants. The activities of the anti-oxidant enzymes (Catalase and ascorbate peroxidase (APX) enzymes) were also improved in the AM inoculated salt treated plants as compared to the control treatments. It was suggested that specific Arbuscular Mycorrhizal Fungi could be used in reducing salt stress in *Pisum sativum* plants.

**O.5.4. IMPACT OF HARVEST MATURITY ON BIOCHEMICAL COMPOUNDS,
ANTIOXIDANTS AND SENSORY CHARACTERISTICS OF KINNOW MANDARIN
FRUIT UNDER EXTRA DOSES OF POTASSIUM AND
PHOSPHORUS NUTRIENTS**

Mahmood Ul Hasan¹, Aman Ullah Malik¹, Raheel Anwar¹, Basharat Ali Saleem²,
Muhammad Shahid³, Muhammad Suliman Shah¹

¹Postharvest Research and Training Centre, Institute of Horticultural Sciences, ³Department
of Biochemistry, University of Agriculture, Faisalabad.

² Punjab Agriculture (Extension) Department, Fruit and Vegetable Development Project,
Sargodha, Pakistan.

Corresponding author Email: mahmoodulhassan1947@gmail.com

Kinnow mandarin is the prime citrus cultivar of Pakistan having pleasant taste and high nutritional value. Fruit quality (external and internal) is an important criteria particularly for export markets. Present study was aimed to evaluate the effect of phosphorus and potassium nutrients on quality of fruit and optimize its harvest maturity with maximum properties. Young Kinnow trees were treated with extra doses (600g and 1200g) of potash and phosphorus contained fertilizers at the time of fruit setting. Fruits were assessed for external as well as internal quality parameters at three times of fruit maturity (fifteen days' interval). Second harvest gave better results in terms of all parameters while, biochemical attributes (TSS, TA %, ascorbic acid, pH) were higher in potash treated treatments as compared to control (farmer practice). Fruits were presented to the panel of judges for sensory evaluation (taste, aroma, flavour and texture) at three times of harvesting; all sensory characters were found to be improved in all treated plants and taste with maximum score at third harvest. However, phytochemicals; antioxidant activity (DPPH %) and total phenolic contents (mg GAE/ 100 mL) increased up to second harvest while declined in third harvest. In conclusion, fruit quality (biochemical, phytochemicals) was improved with potash treatment followed by phosphorus as compared to control. Furthermore, second time of harvest gave best results, with delayed harvesting nutritional profile started declining.

O.5.5. CHARACTERIZATION OF LARVICIDAL ACTIVITY OF BIOGENIC NANOPARTICLES

Neeha Ghafoor¹, Sheeraz Ahmad¹, M Javaid Asad¹, Kumail Ali Rizvi², Tayyaba Zainab¹

¹University Institute of Biochemistry and Biotechnology, Pir Mehr Ali Shah -Arid Agriculture University Rawalpindi.

Corresponding author Email: neehaghafoor@gmail.com

Synthesis of green nanoparticles (NPs) is a one of the most emerging field of Nano biotechnology. Plant extracts are used for the synthesis of green NPs. Green silver nanoparticles are used as vector mosquito control agent against *Aedes aegypti*, vector of Dengue. Silver NPs are more effective than Gold or any other metal because they have property of high stability. AgNO₃ solution was mixed with appropriate amount of Crude Methanolic Extracts (CME) of *Eucalyptus globulus* and *Curcuma longa* to form green AgNps with vigorous shaking. Characterization of these green AgNps is done with U/Visible Spectroscopy for confirmation of silver nanoparticles and Scanning Electron Microscope (SEM) to analyze the nanoparticle size and shape. Both samples showed absorbance between 420-430nm. SEM results showed spherical shape of green silver nanoparticles. The size of nanoparticles of *Eucalyptus globulus* is approximately is 25nm and the nanoparticles of *Curcuma Longa* are of 15nm. These Green NPs were proved to be very effective against 3rd and 4th larval stage of *Aedes aegypti* as they are eco-friendly, less harmful and have strong larvicidal activity. Overall AgNPs of both plants exhibited significant larvicidal activity.

Keywords: Green synthesis, Nanoparticles, Dengue, Larvicidal, Characterization, Silver

O.5.6. A STUDY OF PARAPINOPSIN IN AMPHIBIANS

Tayyaba Zainab¹, Kumail Ali Rizvi¹, S.M. Saqlan Naqvi², Amer Qureshi¹,

Mark Sanderson¹ and Peter Eagles¹

King's College London, United Kingdom.

Corresponding author Email:tzawan@gmail.com,

Parapinopsin (PNP) is a novel non-visual pigment protein found in lower vertebrates. PNP protein is similar in function to the human visual pigment protein rhodopsin, found in the eye. The aim of project was to characterize PNP in different frogs species to understand more about PNP as little is known about its structure and function. Tadpole cDNA from different frogs species (*X.laevis*, *Rana temporaria* and *Rana tigrina*) were used during the project. The cDNA sequencing results of *X. laevis* showed 100% homology but *R. temporaria* and *R. tigrina* showed ~99.9% similarity with *X. tropicalis*. The 0.1% dissimilarity is due to a point mutation from G to A at the 2nd position in the 156th codon. Genomic DNA sequence study results revealed a heterozygosity in *R. temporaria* and a point mutation in *R. tigrina* PNP. This point mutation is very important as it creates a stop codon possibly resulting in protein truncation. Western Blotting results support the cDNA study results with a 37 kDa protein band of PNP in the *X. laevis* protein sample only. Bioinformatics softwares were used to study evolutionary genetics and to predict the 2D structural elements and a 3D structural model for PNP from *X. laevis*. For protein expression studies the coding sequence of PNP gene was chemically synthesized and cloned into the vector pASKGPCR for expression in *E. coli*. The aim of this study was to overexpress and purify the protein for functional studies as well as structural studies using X-ray crystallography and high field NMR.

O.5.7. COCONUT WATER AS SOURCE OF CYTOKININS FOR MICROPROPAGATION OF PLANTS

Fehmina Jabeen, Ambreen Ahmed* and Aqsa Tariq

Department of Botany, University of the Punjab, Quaid-e-Azam Campus,

Lahore 54590, Pakistan

Corresponding author Email: ambreen.botany@pu.edu.pk

Plant biotechnology provides innovative tool for large scale production of horticulturally important plants via various tissue culture techniques. In vitro multiplication facilitates the production of genetically identical plants. Bougainvillea glabra is known for its bright colored showy flowers therefore, largely used in landscaping. Present research deals with in vitro multiplication of Bougainvillea glabra on MS medium and use of these in vitro multiplied plants as a continuous source of explant for callogenesis. Various concentrations of coconut milk with 2,4-D were investigated for callogenic response. In addition, biochemical analysis for protein content, peroxidase activity and auxin content were carried out with naturally growing, in vitro growing and callus of B. Glabra. Extracted proteins were then analyzed through SDS-Polyacrylamide gel electrophoresis. Cell suspension cultures were also grown to carry out cell count. Our findings revealed that coconut milk with 2,4-D showed better callogenic response using nodal and leaf explants. Rate of callus proliferation varies with the changing concentrations of growth regulators, emphasizing the use of best concentrations and combinations of growth regulators for large scale in vitro multiplication of Bougainvillea glabra. Biochemical analysis of naturally grown, in vitro grown and calli of B. Glabra showed that exogenous hormonal concentrations affect the auxin and protein content as well as peroxidase activity.

Keywords: Bougainvillea glabra, callogenesis, coconut water, cytokinins, micropropagation, 2,4-D

O.5.8. PLANT BASED VACCINES: PROMISING CANDIDATES FOR THE PREVENTION OF VARIOUS POULTRY DISEASES IN FUTURE

Zeshan Zulfiqar*, Shahid Ur Rehman, Sidra Bashir, Khizar Hayat, Muhammad Khalid

Bashir, Umar Farooq, Muhammad Ashraf, Muhammad Farooq Khalid

University of Agriculture Faisalabad, Sub Campus Toba Tek Singh

Centre for Applied Molecular Biology & Forensic Sciences University of the Punjab

Corresponding author Email: 4zeshan@gmail.com

Plant-based vaccines are now new way to tackle some of the major threats that are being linked with traditional vaccines in some developing countries. Plant-based vaccines are of recombinant categories in which immunogenic genes are used and extracts of transgenic plants are administered to the birds orally producing antibodies in large quantity against a particular disease. Plant-based oral vaccines are advantageous and economical because of low manufacturing cost. Due to temperature stability they do not need to be cold stored and can easily be stored up to a year in lyophilized form. Their oral route of administration is more convenient for animals and poultry and plant expressed vaccine antigen in maize, rice, soybean and potato can be given as part of feed for animals with adjuvant priming. Bio-encapsulated plant-based vaccines can be used to deliver multiple antigens. Unlike traditional vaccines they are free of associated toxins and pathogens and can easily be scaled up for large production. It can induce mucosal immune response and have higher immune response than traditional vaccines. A number of poultry and animal's disease related recombinant bacterial and viral vaccines have been successfully expressed in plants.

Keywords: Plant Based vaccines, Immunity, Transgenic Plants

**O.5.9. EFFECT OF MORINGA OLEIFERA LEAF SUPPLEMENTATION ON
HEMATOLOGICAL PARAMETERS AND IMMUNE RESPONSE IN
COMMERCIAL BROILER CHICKENS**

Asif Ali, Shaukat Ali Bhatti*, Shahzad Ashraf

Institute of Animal and Dairy Sciences, University of Agriculture,
Faisalabad 38040, Pakistan

Corresponding author Email: sabhatti60@gmail.com

The objective of this study was to evaluate the effect of Moringa oleifera leaf powder on hematological parameters and immune response in broilers. For this purpose, a basal iso-nitrogenous (CP: 20 %) and iso-caloric (ME: 3000 kcal/kg) diet was formulated to act as negative control. The basal diet was supplemented with 50 ppm of zinc bacitracin (positive control), or with Moringa oleifera leaf powder at rate of 1.5 (MLP-1.5), 2 (MLP-2) or 2.5% (MLP-2.5), respectively. Two hundred fifty day-old chicks were divided into twenty five replicates of 10 birds each and distributed to all five treatments randomly. The birds were fed experimental diets from day 3-42. Antibody titers against Newcastle disease was tested using Hemagglutination Inhibition test on day 12th, 23th, and 39th. Blood samples were collected for determination of hematological parameters (PCV and Hb) on day 23rd and 39th. Data were analyzed with GLM procedure of SAS using completely randomized design and means were compared by using Tukey's test. Hemoglobin and packed cell volume on day 23rd was not affected ($P > 0.05$) by dietary treatments. Packed cell volume was higher in positive control than MLP2.5 ($P < 0.05$) at day 39th. Geometric mean values of the antibodies titer against Newcastle disease at 12, 23 and 39 day ranged from 54.4-64, 41.6-128 and 70.4-121.8 respectively, and were not affected ($P > 0.05$) by dietary treatments. It can be concluded that supplementation of Moringa oleifera leaf powder in broiler's diet did not affect hematological parameters and immune response in commercial broiler chickens.

Keywords: Moringa oleifera leaf, hematological parameters, immune response, broiler chickens

O.5.10. PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EVALUATION OF POMEGRANATE PEEL EXTRACT

Mahwish Safdar^{*a}, Summar A. Naqvi^{*a}, Muhammad J. Jaskani^a, Iqrar A. Khan^b, Walliullah^a,
Imran Pasha^c, M. Shahid^d, Asif Hanif^e,

Pomology Lab, Institute of Horticultural Sciences, ^bCenter for Advance Studies, ^cCenter for
Advance Studies, ^dInstitute of Food Science and Technology, ^eDepartment of Chemistry,
^fCenter for Advance Studies, University of Agriculture, Faisalabad 38040.

Corresponding author Email: mahwishsafdar1433@gmail.com; summar.naqvi@uaf.edu.pk

Pomegranate (*Punica granatum* L.) is very important and commercial fruit crop; extensively cultivated in parts of Asia, North Africa, the Mediterranean and the Middle East. In Pakistan, Punjab and KPK are producing Pomegranate in small amount but the dominant region for pomegranate is Baluchistan. Pomegranate peel that is left over after juice is very important for human health. Pomegranate peel has antioxidant and antimicrobial activities due to ellagic acid. The objective of the study was to identify the phytochemicals, antibacterial and antioxidant ability of two Afghanistan varieties of pomegranate peel extract i.e. Spin Trush and Turosha Taki. Turosha Taki variety showed highest antioxidant activity (49.07 %), total phenolic contents (132.72 mg GAE/100g) and antimicrobial activity against four bacteria i.e. *Bacillus subtilis* (40.5cm), *E.coli* (119.12cm), *Pasteurella multocida* (18.82mm) and *S.aureus* (15.65mm). GC-MS of Spin Trush and Turosha Taki of n-hexane extract of pomegranate peel showed 18 and 28 peaks on chromatogram. The major compound of Spin Trush Amino propionic acid 48.12% and the Turosha Taki was showed Hexasiloxane 48.32%. FTIR identified volatile compounds of Spin Trush and Turosha Taki with wavelength 616.16 cm⁻¹ and 3317.08 cm⁻¹. It was concluded that the Spin Trush and Turosha showed high value of antioxidant, TPC and having antimicrobial activity against microbes. It was also observed that the Spin Trush and Turosha Taki using GCMS and FTIR was an extremely effective technique for determining the presence or absence of variability of phytochemical and volatile compounds.

Keywords: *Punica granatum*. Antimicrobial activity, Antioxidant activity, Phenolic, GC-MS, FTIR

O.5.11. MERCAPTO-TRIAZOLE PRIMING FOR ALLEVIATION OF CADMIUM TOXICITY IN WHEAT

Arruje Hameed¹, Amjad Hameed², Shamila Naz³, Matloob Ahmad⁴, Tahir Farooq^{3,*}

¹Department of Biochemistry, Government College University, Faisalabad, Pakistan

²Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang road Faisalabad, Pakistan.

³Department of Applied Chemistry, Government College University, Faisalabad, Pakistan.

⁴Department of Chemistry, Government College University, Faisalabad, Pakistan.

Corresponding author Email: tahirfarooqfsd@gmail.com

Cadmium interferes with the calvin cycle, pentose phosphate pathway and biosynthesis of chlorophyll. It reduces soluble proteins, sugars, phenolic contents and antioxidant enzyme activities. Cd induced changes in biochemical attributes results in to physiological, biochemical and genetical disorders in germinating plants. Various natural and synthetic priming agents had been employed as stress management strategy. We have used triazolic compounds as priming agents to evaluate their effects on biochemical attributes in wheat seedlings under Cd stress (5 μ M). The wheat seeds were primed with 10, 20, 30 and 40 ppm of each triazole. In wheat seedlings, protein contents, SOD, POD, CAT, ascorbate peroxidase, MDA, esterase, protease, TPC, TOS, ascorbic acid, α -amylase and chlorophyll contents were studied under normal and stress conditions and compared with control. Triazole-priming helped to reduce the negative impact of Cd stress on aforementioned biochemical attributes. Almost all triazole treatments induced significant improvement in protein contents, POD, SOD and esterase activity under stress conditions. Priming with different concentrations of triazoles increased CAT, α -amylase activities and TPC contents. All priming treatments significantly reduced the MDA contents. Increase in aforementioned biochemical attributes along with a decrease in MDA contents represent a mitigating effect of triazole priming on Cd toxicity in wheat seedlings.

Keywords: Seed priming; antioxidant enzymes; triazole-priming; Cd stress; wheat

**O.5.12. EFFICACY OF HEARTWOOD EXTRACTIVES OF *ALBIZIA LEBBECK* (L.)
BENTH. AGAINST SUBTERRANEAN TERMITES**

Sohail Ahmed, Sara Kushf, Babar Hassan, Uzair Saleem

Department of Entomology, University of Agriculture, Faisalabad.

Heart wood extractives of *Albizia lebbbeck* (L.) Benth. (sirin kala) in four solvents (n-hexane, pet ether, ethyl acetate and water at four concentrations (0. 2.5, 5 and 10 mg ml⁻¹) were tested against subterranean termites to compare mortality, antifeedant/repellency and field protection of poplar wooden stakes treated with these extractives. Highest concentration of ethyl acetate extractive proved to be toxic and repellent to termites significantly when compared with other extractives. Poplar wooden stakes were also protected in field exposure to the termites treated with extractive of ethyl acetate either dip or vacuum pressure application methods. Organic solvent extractives imparted significant low weight loss percentage than respective control and water extractives in dipping and vacuum application. Ethyl acetate protected >2 fold as compared to water and other extractives significantly.

Keywords: Extracts, *Albizia lebbbeck*, Termites, Organic solvents

O.5.13. AZO DYES DERIVATIVES FOR ORGANIC PHOTOVOLTAICS

Dr Shafiq-ur-Rehman^a, Dr. Shamsa Bibi^{*a}, and Mehwish Khan^a.

Department of Chemistry, University of Agriculture Faisalabad^a.

Natural azo compounds isolated from fungi, plant, bacteria, and invertebrates. More than 120 biologically active diazene containing alkaloids demonstrate confirmed pharmacological activity, including antitumor, antimicrobial, and antibacterial effects. A series of low energy gap cis-trans aminoazobenzene based donor molecules with dipropylamine donating group and different electron withdrawing groups (OH, CN, NH₂, NO₂) are investigated for solar cell applications. These investigated molecules are studied computationally at PBE1PBE/ 6-31+G** level of theory using DMSO solvent with CPCM model. Structural properties, UV-Vis absorption spectra, electronic properties, light harvesting efficiency, exciton binding energy and charge transfer properties of 1-4 Cis-Trans molecules are investigated. 4-Cis and 4-Trans molecules have smaller energy gap of 2.91 and 2.69, respectively. Absorption spectra of these dyes also showed red shift with respect to other investigated molecules and reference molecules at 4-Cis λ_{max} of 542.31 nm and 4-Trans λ_{max} of 528.14 nm. Although investigated molecules are good hole transfer while 2-Trans and 4-Trans are the best hole transfer. In 4-Cis and 4-Trans molecules, bond lengths are increased while bond angles and dihedral angles are decreased which showed that structural properties are affected by changing electron withdrawing groups and all investigated molecules showed approximately π - π^* transitions. According to the results, it is justified that how different electron anchoring groups influence the structural and optoelectronic properties. We suggest that investigated donor molecules are appropriate candidates for high performance organic solar cell devices.

Keywords: Density Functional Theory, Organic Solar Cells, Azo Dyes. Optoelectronic properties.

O.5.14. NMR BASED COMPARISON OF BIOACTIVE COMPOUNDS PRESENT IN HUMAN MILK AND COW'S MILK

Fozia Saleem¹, and David S. Wishart²

¹Department of CABB, University of Agriculture Faisalabad

²Department of Biological Science, University of Alberta, Canada.

Corresponding author Email: fozia@ualberta.ca

Milk is often called the “perfect food” Produced from the mammary glands of all peri-parturient female mammals. Milk is rich in key nutrients such as carbohydrates, proteins, fats, minerals and vitamins that are dynamically adjusted to meet the specific developmental needs of growing newborns. While milk is normally a species-specific bio fluid consumed by young mammals belonging to that species but infants of those women who are unable to continue breastfeeding uniquely consume cow milk as a safe alternative to breastfeeding. There are many bioactive substances present in low concentrations in human milk but absent from bovine milk with proven effect on nutrient utilization or other health benefits. These bioactive substances are unable to detect by normal biochemical techniques. In an effort to both detect and substantially differentiate human and cow milk nutrition, 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 human and cow's milk. In particular, using nuclear magnetic resonance (NMR) and, gas chromatography mass spectrometry (GC-MS), we were able to quantify and validate more than 25 bioactive that are unique to human milk. Some of these bioactive are not only modulate the immune system of premature or mature newborns but also protect them against infections. NMR-based metabolic profiling can provide a rapid characterisation of breast and cow milk composition, thus allowing a better understanding of its nutritional properties to choose the better option.

Keywords: Milk, Bioactive compounds, NMR

O.5.15. MICROWAVE ASSISTED SUSTAINABLE ISOLATION OF COCONUT HUSK BASED NATURAL COLORANT FOR WOOL DYEING

Shumaila Kiran *, Shahid Adeel, Tahsin Gulzar and Sajjad Yusuf

Department of Applied Chemistry, Govt. College University Faisalabad 38000 Pakistan

Corresponding author Email: shumaila.asimch@gmail.com

The natural dyes being used in recent years for dyeing the natural fabric are attributed towards its non-toxic & eco-friendly nature and health concerns. Coconut coir (*Cocos nucifera*) which contains tannin, has been selected for dyeing of wool fabric. Extraction was carried out using aqueous and acidic media. The extracts and fabrics were irradiated for 1, 2, 3, 4, 5 and 6 minutes. To get optimum results, dyeing of treated and un-treated fabrics were carried out using treated and un-treated extracts. For optimum coloring variable, dyeing of fabric was carried out at 35 to 85⁰C, for 35 to 85 minutes using salt concentration of 1-10g/100mLs. For improvement in rating of colorfastness, salts of Iron and Al and Tannic acid as chemical mordants has been employed using optimum extraction and dyeing conditions. All dyed samples were investigated using Spectra Flash SF 600. The results showed that microwave treatment has improved the colour strength onto cotton fabric whereas ISO standard methods for rating of fastness show that chemical mordanting has improved the properties from good to excellent.

Keywords: *Cocos nucifera*, Chemical mordents, Wool, Microwave radiations

O.5.16. HORIZONTAL TRANSMISSION OF *METARHIZIUM ANISOPLIAE* IN ECONOMICALLY IMPORTANT FRUIT FLY SPECIES (*BACTOCERA* SPP.) IN PAKISTAN AND INFECTIVE EFFICACY OF FUNGUS AGAINST SOME BIOLOGICAL PARAMETERS OF MELON FRUIT FLY.

Mubashar Iqbal*, Muhammad Dildar Gogi* and Muhammad Jalal Arif*

*Department of Entomology, University of Agriculture, Faisalabad

Corresponding author Email: mubashariqbal2635@gmail.com

Fruit flies (*Bactrocera zonata*, *B. dorsalis* and *B. cucurbitae*) (Diptera: Tephritidae) have great threat to fruit and vegetables industry in Pakistan. Research experiments were conducted in IPM lab, Dept. of Entomology, UAF, Pakistan. *M. anisopliae* isolate obtained from USDA-ARS, USA. Day conidia were tapered on velvet in plastic tube and cover with lid. Equal number of male and female flies of three species were introduced into treated tube separately for 2 to 4 minutes and transfer into non treated separated cages already field with field with adult diet. Respect non-infested males and females were allowed to enter for mating for 24 hours. Results revealed that, infected males and female fruit fly species were able to transmit infection to healthy fruit flies species. Mortality for *B. dorsalis* after infested donor species (81.54%) after 12 days of post inoculation similarly mortality of *B. zonata* (79.69%) after 12 days while *B. cucurbitae* show low mortality (69.67%) after 14 days. Significantly lower fecundity as compared to the untreated flies were observed in treated *B. cucurbitae*. Mean egg hatch for treated females, recipient females, and untreated females of *B. cucurbitae* was 57.23, 63.34, and 69.35 respectively. Finding suggested that horizontal transmission of fungal infection occur during physical contact and mating and these infection also reduced fertility and fecundity of insect pest. Results also concluded that *M. anisopliae* could be incorporated into SIT programme for the control of *B. zonata*, *B. dorsalis* and *B. cucurbitae*.

Keywords: *B. zonata*, *B. dorsalis* and *B. cucurbitae*, *M. anisopliae*, horizontal transmission,

O.5.17. EVALUATION OF MYCOFLORA ASSOCIATED WITH *PHASEOLUS VULGARIS* IN AJ&K, ITS IMPACT ON GERMINATION AND MANAGEMENT STRATEGIES

Shoukat Hussain¹, Muhammad Ajaib^{2*}, Rehana Asghar¹, Imran Ali¹
and Muhammad Faheem Siddiqui^{3*}

¹Department of Biotechnology, ² Department of Botany, Mirpur University of Science and Technology (MUST), Mirpur-10250 (AJK), Pakistan

³Department of Botany, University of Karachi, Karachi 75270, Pakistan

Corresponding author Email: shoukatch2013@gmail.com.

Seed of *Phaseolus vulgaris* L. collected from different localities of State of Azad Jammu and Kashmir were evaluated for seed associated Mycoflora by standard blotter paper method and Agar plate method. The seed samples were observed contaminated with fungi belonging to eight genera i.e. *Alternaria* (Nees), *Aspergillus*(Micheli), *Curvularia*(Boedijn), *Drechslera* (S. Ito), *Fusarium* (Link), *Mucor* (Fresen), *Penicillium* (Link) and *Rhizopus*(Ehrenb). The highest infection percentage was recorded from Trarkhal, Bagh and Chakaar samples, i.e. 24.75A, 18.25B and 13.25C respectively. Least infection percentage was observed on Lawaat (3.00H) and Kail (11.8E) samples. Impact of fungal presence on seed germination was elucidated by standard rolled paper towel method. Lawaat and Kail samples show better germination 94% and 92% respectively. Incidence of fungi was recorded high and low germination in Trarkhal and Palangi samples 43% and 22% respectively. Three treatments namely, heat, *Allium sativum* extract and Benomyl (fungicide) were used for Mycoflora management. Use of different antifungal treatments found effective in increasing germination percentage and health of seedlings. Efficacy of management was evaluated by using CRD2 Factorial test. The interaction between treatments and localities was significantly different at 0.05 level of significance. Benomyl was more effective in increasing seed germination with 76.37 mean. Locality of Lawaat shows the highest germination percentage with different treatments having 96.00 mean value. Best interaction was observed between treatment 2 and locality 2, i.e. 98.00 while least interaction was calculated between treatment 1 and locality 6 i.e., 15.00. Seed samples of *P. vulgaris* collected from different sites were found contaminated with seed associated Mycoflora. These fungi reduce the germination percentage and use of different antifungal treatments efficiently increases the germination percentage and reduce the fungal growth. Benomyl found more effective for seed treatment before sowing.

**O.5.18. THE EVALUATION OF PRIMARY OR SECONDARY HEADACHE IN
DIFFERENT AGE GROUP BY USING COMPUTED TOMOGRAPHY (CT) AS
IMAGING MODALITY**

Ayesha Tariq¹, Sadia Sana², Naheed Akhter²

College of Allied Health Professionals,

Directorate of Medical Sciences, GCUF

Corresponding author Email: sadidasana203@gcuf.edu.pk

The most common problem is Headache which some people experience it in early ages or some people experience it in old ages but its causes, duration and severity changes person to person. Headache is classified into two main categories. Primary headaches are those which do not have any underlying cause. They occur naturally caused by stress/tension, cluster headache or migraine. They do not require further CT evaluation. Secondary headaches are those headaches that are associated with underlying organic causes or other brain disorders like brain tumor, hypertension, cerebral hemorrhage, cerebral infarction or the cerebral atrophy etc. According to previous studies, about only 10% headaches are secondary headaches when evaluated by neuroimaging. Neuroimaging includes CT/MRI evaluation. Objective of the study was the evaluation of primary or secondary headache in different age group by using computed tomography as imaging modality. All the CT scans related to research were conducted in District Head Quarter Hospital Okara from July 2018-November 2018. Cross section study was conducted on 80-100 patients to evaluate the type of headache either primary or secondary headache in different age group patients by using Hi-tech 16 slice CT scanner. The sampling technique was simple random sampling. History was taken and questionnaire was filled. Scan type was axial. Complementary views were sagittal and coronal. The slice thickness was 4mm. The data analysis was done by using the SPSS latest version. Data was analyzed and the main focus was on the findings causing headache in different age groups. The p-value was considered as the index for the null hypothesis from the observed outcome, the p-value < 0.05 it was considered as statistically significant. The study was conducted on 100 patients presented with headache. Out of 100 patients 22% patients had headache due to hypertension and it was 55% in people of age group 21-40 years. 20% patients were suffering from cerebral atrophy in which 50% people were of age 41-60 years and 40% were of age group 61-80 years and 10% were above 90 years. Hemorrhage was seen in 11% of patients and 63.3% people were of age group 41-60 years. Cerebral infarction was present in 12% of patients and 58.3% people were

of age group 61-80 years. Brain tumor was present in 8% of people and about 50% of people were of age 41-60 years. 15% patients were normal had only stress headache about 60% patients were of age 21- 40 years. Post infection headache was seen in 3% of patients. Post head injury headache patients were 5% Ischemic insult along with cerebral atrophy was present in 1% of patient's. Hydrocephalus and meningitis was seen in 2% and 1% of patients respectively. It is concluded that most prevalent cause of headache in young age group from 21-40 years is hypertension along with vertigo or vomiting in some cases. Stress is the second cause of headache in young people. Cerebral atrophy is the most prevalent cause of headache in people of age above 40 years. Cerebral infraction and hemorrhage are second most prevalent causes of headache in age above 40 years.

Keywords: *primary headache, secondary headache, age group, computed tomography*

**O.5.19. STUDY OF ANTIBACTERIAL POTENTIAL OF THUJA ORIENTALIS
LEAVES ESSENTIAL OIL COLLECTED FROM SEMI-ARID CLIMATE**

Rafia Rehman*, Muhammad Asif Hanif, Muhammad Shahid, Afsar Bano

Corresponding author email: rehman_rafia@yahoo.co.uk

Thuja orientalis (L.), belonging to family Cupressaceae, is a marvelous, evergreen plant which has been used anciently for its medicinal importance. In the present study, the essential oil of *Thuja orientalis* (L.) Franco leaves, collected from semiarid climate, was extracted by hydrodistillation. The extraction conditions were optimized in order to get considerable yield of essential oil (0.1%). Initial analysis of these oils by TLC showed the presence of different polarity groups ranging from non-polar terpene hydrocarbons to polar terpenoid alcohols. GC-MS analysis revealed that major component of hydrodistilled essential oil of *Thuja orientalis* (L.)Franco leaves was α -pinene (17.8%). The antibacterial potential of this essential oil was evaluated against gram positive and gram negative bacterial strains. The results showed that hydrodistilled essential oil of *Thuja orientalis* is slightly more active against *S. aureus* as compared to *E. coli*. The present study emphasize that leaves essential oil of *Thuja orientalis* (L.) can be used to develop natural antibacterial agents.

Keywords: *Thuja orientalis* (L.), semi-arid climate, essential oil, GC-MS, antibacterial activity

**O.5.20. EVALUATION OF ANTIFUNGAL POTENTIAL OF LEAF EXTRACT OF
CHENOPODIUM MURALE AGAINST *FUSARIUM OXYSPORUM* F. SP.
*LYCOPERSICI***

*Arshad Javaid¹, Syeda Fakehha Naqvi¹, Muhammad Zahid Qureshi²

¹Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

²Department of Chemistry, GC University, Lahore, Pakistan.

Corresponding author Email: arshad.iags@pu.edu.pk, arshadjpk@yahoo.com

The present study was performed to evaluate antifungal activity and GC-MS analysis of leaf extract of *Chenopodium mural* against *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a highly problematic soil-borne pathogen of tomato. Dried leaves of *C. murale* were extracted with methanol for 2 weeks and after evaporating the solvent on a rotary evaporator, antifungal bioassay was carried out against FOL. All concentrations (1% to 5%) significantly reduced FOL biomass by 14–45%. The remaining methanolic extract was fractioned with n-hexane, chloroform and ethyl acetate and all these fractions were assayed for their antifungal potential. A 200 mg mL⁻¹ concentration of various sub-fractions reduced fungal biomass significantly by 94-98% over control. All the sub-fractions were subjected to GC-MS analysis that revealed presence of 32 compounds in n-hexane, 2 compounds in chloroform and 13 compounds in ethyl acetate sub-fraction. The predominant compounds in n-hexane sub-fraction were hexadecanoic acid, methyl ester (14.64%), methyl linolenate (16.61%) and γ -sitosterol (13.53%). In chloroform sub-fraction, bis (2-ethylhexyl) phthalate (92.31% and in ethyl-acetate sub-fraction, ethyl butyrate (19.57%), dihexyl phthalate (11.19%) and dioctyl phthalate (12.16%) were present in higher concentration.

POSTER PRESENTATIONS

P.1. PATHOPHYSIOLOGICAL ROLE OF GLUCOCORTICOID RECEPTOR (GR) LIGANDS IN ACETAMINOPHEN INDUCED LIVER INJURY IN MICE

Mehwish Batool¹, Bilal Aslam^{1*}, Faqir Muhammad¹, Junaaid Ali Khan¹, Sultan Ali²
and Asif Hussain¹

¹Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Agriculture
University Road, Faisalabad, Pakistan. Zip Code 38000

²Institute of Microbiology, University of Agriculture, Faisalabad-38000-Pakistan

Corresponding Author email: cba933@gmail.com

Stress elicited by various drugs and psychosocial components is extensively prevalent globally. Glucocorticoid receptors (GR) control different features of lipid metabolism and lipoproteins in liver. Acetaminophen (APAP) is broadly used antipyretic and analgesic drug. Overdose of acetaminophen causes centrilobular necrosis and fibrosis by the generation of oxidative stress. Twenty-five mice were divided into five groups. Hepatoprotective effects of GR-ligands i.e. Mifepristone (RU-486) and Dexamethasone (DEX) were examined in albino mice hepatotoxic models. Liver injury biomarkers i.e. Aspartate Aminotransferase (AST) and Alanine transaminase (ALT), and oxidative stress markers i.e. Total antioxidant capacity (TAC) and Total oxidant status (TOS) were analyzed by the assistance of biochemical kits and automated biochemistry analyzer. The data was analyzed statistically by using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range (DMR) test. This experimental study was conducted to evaluate alone as well as combined systemic effects of GR-ligands, mifepristone and dexamethasone on oxidative damage and liver injury induced by acetaminophen. Evaluation of parameters of liver functions showed substantial increase in ($P<0.05$) AST, ALT levels accompanied by elevation ($P<0.05$) in TOS and reduction ($P<0.05$) in TAC in APAP-induced hepatotoxic group. While the groups received dexamethasone, mifepristone and dexamethasone + mifepristone as pretreatment significantly ($P<0.05$) decreased the levels of ALT, AST and TOS as compared to toxic group (APAP). The protective effects of glucocorticoid receptor ligands in APAP-induced liver injury were further verified by histopathological examination. However, additional studies are necessitated to explicate completely the hepatoprotective efficacy, safety profile, basic underlying process of protection and therapeutic applications of glucocorticoid receptor ligands.

Keywords: Glucocorticoid receptor ligands, Acetaminophen, Mifepristone, Dexamethasone, Oxidative stress, Hepatotoxicity

P.2. LABORATORY-ACQUIRED INFECTIONS AND BIOLOGICAL RISKS MITIGATION IN HEALTH BIOTECHNOLOGY

Ghulam Rasool^{1*}, Muhammad Riaz¹, Akhtar Rasul¹, Arif Muhammad Khan²

¹Department of Allied Health Sciences, Sargodha Medical College, ²Department of Biotechnology, University of Sargodha, Sargodha-Pakistan.

Advancements in biotechnology research have broader applications in a variety of disciplines like microbiology, biopharmaceuticals, agriculture, food and nutritional sciences, genetically modified organisms and nanotechnology etc. Although, research with pathogenic agents are of greater concern due to their potential risks for both the living and the surrounding environment. Biosafety and biosecurity measures are crucial in biotechnology laboratories for the protection of staff and the environment being exposed to hazardous agents. Biosafety training programs are essential for the researchers and other laboratory personals to get understand the handling procedures of hazardous biological agents. The biological risks can be controlled by the correct application of standard operating procedures (SOPs) international recognized for biosafety measures. The biosafety SOPs like proper hand washing techniques, proper containment apparatus, beaking methods, donning and doffing of personal protective equipments (PPEs), responding to spills, needle stick injuries, shipment and decontamination of infectious substances and proper waste disposal. Proper training of biosafety, biosecurity and adherence with biosafety standards guidelines may prevent or decrease the biological risk. The scientific community may benefit from biosafety training sessions, workshops and lectures. The aim of this study is to highlight the increase in laboratory acquired infections and the mitigation of biological risks by adopting the internationally recognized guidelines for biosafety and biosecurity.

Keywords: laboratory-acquired infections, biological risks, biosafety, biotechnology

P.3. TREATMENT OF CANCER PATIENTS BY METHYLGLYOXAL BASED-FORMULATION

Awais Ali^{*1} and Sana Javeed.

¹Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan,

² Department of Chemistry, Islamia University Bahawalpur, Pakistan.

Corresponding author Email: awaismalik0306@gmail.com

A historical prospective on methylglyoxal research is presented, mentioning the document anticancer and antiviral effects of methylglyoxal. Methylglyoxal act against number of pathogenic microorganisms. methylglyoxal has important role in host organisms. Several in vitro and in vivo studies showing that methylglyoxal act specifically against different type malignant cells. The idea and supporting experimental evidence of Albert Szent-Gyorgyi, J. Halder, M. Ray and S. Ray Int. J. Cancer 1993. This methylglyoxal is growth regulator and can act as anticancer. This formulation has tumoricidal effect by inhibiting in cancerous cells the electron flow and transfer of reducing equivalent necessary for production of adenosine-5-phosphate. The cellular energy currency by keeping remarkable property in mind, we have treated 21 patient in age group 29-58 YRS. Which suffering from different type of malignancy with this methylglyoxal-based formulation. Each patient received by orally at a time maximum 10ml of 0.4-0.5 M methylglyoxal dilute in 50-60ml of water. This result indicates showing positive effect on all patient. 10 out of 21 patient treating under excellent, physical condition of 6 patient can be considered stable opted out from the treatment or died during the course of study. The symptomatic improvement of many patient who died of progressive disease suggest that formulation could be used for palliation.

Keywords; Adenosine tri phosphate, anti Cancer, methylglyoxal, antiviral.

P.4. EVALUATION OF BIOACTIVE FRACTIONS EXTRACTED THROUGH ORGANIC AND AQUEOUS SOLVENT FROM *HAMELIA PATENS* LEAVES

Iram Amir and Zahid Mushtaq

Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: iramparacha676@yahoo.com, zahid mushtaquaf@uaf.edu.pk

Plants have been used as medicines for many years. Secondary metabolites and other bioactive compounds are collected from plant materials and used for beneficial medicinal effects. The biological activities found in plants are shown outstanding to the attendance of bioactive compounds like alkaloids, tannins, steroids, terpenoids, phenols and glycosides. Various aqueous and organic solvents are used for extraction of bioactive compounds. *Hamelia patens* are used in the handling of many conservative drugs and infectious diseases, which belongs to the plant family Rubiaceae. The present research is concerned to investigate the bioactive potential of *Hamelia patens* leaves extract by using different solvents in order manner. Bioactive prospective of *Hamelia patens* leaves extract was checked by performing different assays for evaluating their antimicrobial, antioxidant, hemolytic, thrombolytic and enzyme inhibitory activities done. Crude methanol extract show maximum antimicrobial activity. The maximum antioxidant activity was exhibited by chloroform fraction (39%). Chloroform fraction exhibited maximum α -amylase inhibition (67%). Lipase inhibitory activity crude methanol extract show maximum activity (32%). Water soluble fraction showed minimum hemolytic activity (2%). The most bioactive extracts were being performed by using high performance liquid chromatography (HPLC). Crude methanol extracts showed the presence of Quercetin (0.4079 mg/g), syringic acid (12.227 mg/g), *M*-coumaric acid (0.5943 mg/g) and Sinapic acid (2.0178 mg/g). From the other chloroform solvent extracts it was observed that Quercetin (0.351 mg/g), *p*-coumaric acid (1.179 mg/g), syringic acid (1.508 mg/g), and ferulic acid (5.25 mg/g) were present. In absolute ethyl acetate extract, gallic acid (0.248 mg/g), vanillic acid (3.95 mg/g), sinapic acid (3.793 mg/g) and Quercetin (0.456 mg/g) is present. Their results show the maximum activity was exhibited all activities. Results were analyzed by using (ANOVA) one-way analysis of variance.

P.5. BIOETHANOL PRODUCTION FROM VARIOUS WASTE PAPERS

Muhammad Kashif Javed

Corresponding author Email: kashifjaved25443@gmail.com

The production of bioethanol from various waste papers (newspaper, office paper, magazine and cardboard) was evaluated from an environmental standpoint. ‘Cradle-to-grave’ (or ‘well-to-wheel’) analyses were performed using a Life Cycle Assessment (LCA) approach with the aims of identifying the key drivers of environmental impact in the bioethanol supply chains and of comparing the environmental footprints of various bioethanol supply chains with those of conventional petrol. Base cases (bioethanol production from various waste papers) and two state-of-the-art cases including pre-treatment of office paper by dilute acid (DA) and of newspaper by an oxidative lime (OL) process were constructed using laboratory data, expert consultations, literature values, and simulation in Aspen Plus™ software. Contribution analysis showed enzyme production needed for hydrolysis of the papers to be the main contributor to the environmental profiles for bioethanol in the base cases. The production of process heat and hydrochloric acid respectively were the main contributors to the bioethanol environmental profiles for office paper-to-bioethanol with DA pre-treatment and newspaper-to-bioethanol with OL pre-treatment. Overall, bioethanol produced from newspaper, magazine paper and cardboard were found to have a lower environmental impact than the conventional transport fuel petrol. However, this conclusion is significantly affected by the system boundaries used for the analysis. When an expanded system boundary is applied to consider virgin and recycled paper production as the potential consequential effects within the bioethanol and petrol systems respectively, office paper derived bioethanol systems emerge as the most environmentally favorable over petrol.

P.6. PHARMACOKINETIC AND BIOAVAILABILITY STUDY OF ORAL SITAGLIPTIN IN HEALTHY ADULT FEMALE SUBJECTS

Bilal Aslam^{1*}, Maida Rizwan¹, Faqir Muhammad¹, Mashkooor Mohsin² and Asif Hussain¹

¹Institute of Pharmacy, Physiology and Pharmacology, ²Institute of Microbiology, University of Agriculture, Faisalabad-38000-Pakistan

Corresponding Author email: cba933@gmail.com

In the current study, influence of genetic dependence and environmental conditions of sitagliptin on the pharmacokinetics in healthy adult female subjects was determined. Ten female subjects of weight 48-60 and age 25-35 years old were selected. Sitagliptin is being currently used in human clinics for treatment of type 2 diabetes. A single dose of 50mg of sitagliptin was administered orally to subjects and the blood samples were collected at different intervals of time such as on 0.5, 1, 2, 3, 4, 8, 10, 12 and 24hour post medication. Centrifugation of blood samples were done to separate the plasma and stored at -20°C. Drug concentrations in plasma were determined by using HPLC technique. By following one compartment open model, plasma concentration verses time data was used to compute pharmacokinetic parameters using computer software program (APO). Mean±SD elimination half-life of sitagliptin was 13.249±0.9257 (hours) in healthy female subjects after single oral dose. The value for volume of distribution was 2.2873±0.098234 L/Kg. Maximum plasma concentration of drug was 0.28908±0.014814317µg/mL which was attained after 3 hours of drug administration. The value of total body clearance was 0.27319±0.005571. In female human subjects, pharmacokinetic parameters of sitagliptin like $t_{1/2\beta}$, V_d remain higher than most respective reported values, while CL_B remain lower than most of the studies. Bioavailability parameters of sitagliptin e.g. C_{max} , T_{max} are lower than the values in most of the foreign studies whereas AUC values are higher than most respective values in the foreign counterparts. These pharmacokinetic variations show environmental variations. C_{max} , T_{max} , $t_{1/2}$, AUC, CL_B , and V_d of sitagliptin in healthy female subjects are different as compared to their respective values reported in the literatures and this difference is may be due to gender or age differences. Various factors like change in genetic morphology, dietary habits and local environmental conditions may influence on pharmacokinetic parameters.

KEYWORDS: Pharmacokinetic study, Environmental effects, Sitagliptin, Hypertension

P.7. EFFECT OF CURCUMIN PRE-TREATMENT ON PHARMACOKINETICS OF VALSARTAN IN ALBINO RATS

Bilal Aslam^{1*}, Aniza Ashraf¹, Faqir Muhammad¹, Mashkooor Mohsin² Asif Hussain¹

¹Institute of Pharmacy, Physiology and Pharmacology, ²Institute of Microbiology, University of Agriculture, Faisalabad-38000-Pakistan

Corresponding Author email: cba933@gmail.com

Valsartan is an antihypertensive drug which is widely used for lowering of blood pressure. Cadmium chloride is administered intra-peritoneal to induce hypertension by direct contraction of vascular smooth muscle in albino rats. The aim of current study is to evaluate whether curcumin capable to alter pharmacokinetic parameter of valsartan and to determine the synergistic effect of curcumin and valsartan in hypertension. Valsartan 10mg/kg was administered in rats after repeated oral doses of 100mg/kg of curcumin for 7 days in treated group and same dose of valsartan will be administered in the normal (control) group only. After 7 days, blood samples were collected to study the pharmacokinetic parameters of valsartan and during curcumin administration. Further assessment of interaction was considered. Peak plasma concentration C_{max} of valsartan was 30.8 ± 1.98 mg/L when it administered alone while valsartan administration along with curcumin increased the C_{max} to 37.5 ± 2.91 mg/L. Curcumin significantly ($P < 0.05$) increased AUC of valsartan from 117 ± 4.59 to 173.6 ± 16.6 mg.h/L when both drugs concurrently administered to hypertensive male rats. The clearance (CL_B) of valsartan non-significantly ($P > 0.05$) decreased from 0.01 ± 0.004 to 0.008 ± 0.001 L/h/kg on concomitant administration with curcumin. Curcumin non-significantly ($P > 0.05$) decreased the volume of distribution (V_d) of valsartan from 0.032 ± 0.009 to 0.020 ± 0.003 L/kg due to presence of interaction between herb and drug.

Keywords: Valsartan, Hypertension, Curcumin, Drug interaction

**P.8. PHYTOCHEMICAL ANALYSIS AND GASTROPROTECTIVE POTENTIAL OF
RICINIUS COMMUNIS AGAINST NSAID INDUCED ULCER IN
ADULT RABBITS**

Muhammad Usman Bari¹, Bilal Aslam^{1*}, Faqir Muhammad¹, Sultan Ali² and Asif Hussain¹

¹Institute of Pharmacy, Physiology and Pharmacology, ²Institute of Microbiology, University of Agriculture, Faisalabad-38000-Pakistan

Corresponding Author email: cba933@gmail.com

Ricinus communis also known as “Castor” is widely used in traditional medicine due to its various pharmacological activities. The current study is designed to observe its efficacy in NSAID induced gastric ulcer in animal model. For this purpose, *R. communis* leaves were collected, dried and subjected to ethanolic extraction. Phytochemical analysis was performed to detect various phytochemicals present in castor leaves. To observe gastroprotective activity, healthy adult rabbits were selected and were divided into six groups (n=6). Normal control group was on routine diet. Gastric ulcer was induced in other all groups by giving Aspirin (150mg/kg/day, orally). Second group used as disease control group, was on Aspirin (150mg/kg/day, orally) only. Ranitidine (20mg/kg/day, orally) was given as standard gastroprotective drug while three ulcer induced groups received different doses of *R. communis*. 14 days trial was conducted. At the end of experiment, stomachs of all rabbits were collected to evaluate gastroprotection by checking gastric pH and volume, total acid output and Ulcer score. Oxidative parameters were determined from serum samples. One-way ANOVA was applied on obtained data followed by Duncan’s Multiple Range (DMR) test for statistical analysis. Phytochemical analysis showed the presence of carbohydrates, alkaloids, flavonoids, phenolics and glycosides. Results showed significant ($P<0.05$) decrease in total acid output, gastric volume, ulcer index and ulcer score in *R. communis* treated group while gastric pH raised to normal range. *R. communis* treatment reduced serum oxidative stress parameter (TOS and MDA) and raised antioxidant level (TAC and Catalase) significantly ($P<0.05$). Histopathological studies showed ulcer prevention in *R. communis* (450mg/kg/day) group as comparative to ranitidine treated group. Above results showed that *R. communis* is potential gastroprotective plant.

Keywords: *Ricinus communis*, Gastric ulcer, Gastroprotection

P.9. Evaluation of the comparative anti-diabetic efficacy of *Ocimum sanctum* and metformin in alloxan diabetic rats

Bilal Aslam^{1*}, Tayyeba Ramzan¹, Faqir Muhammad¹, Asif Hussain¹
and Zia-ud-Din Sandhu²

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Parasitology,
University of Agriculture, Faisalabad-38000-Pakistan

Corresponding Author email: cba933@gmail.com

Diabetes is a group of metabolic disorders which consist of defective insulin secretion, action of insulin on body, or both may occur. Metformin is commonly used to treat of diabetes to lowers the blood glucose concentrations. In this experimental study the hypoglycemic effect of alcoholic leaves extract of *Ocimum sanctum* and metformin was checked in diabetic rats. The experimental protocol for 0-21 day was designed, which consist of 5 groups and each group consist of 6 rats. 1st Group was control group, receiving routine diet and ad-libitum water, 2nd Group was treated with Alloxan (120mg/kg i.p) to induce diabetes. 3rd Group was control group on metformin (100mg/kg/day). 4th Group; treated group-1 on alcoholic extract of *Ocimum sanctum* (50mg/kg/day) and 5th; treated group-2 combination of *Ocimum sanctum* (50mg/kg/day) and (100mg/kg/day) metformin. Different parameters such as Blood glucose IU/ml, fasting sugar, postprandial glucose level, leptin, amylase, GCK and HBA1C was performed while liver function test (ALT, AST), Serum lipid profile (Serum triglycerides, total cholesterol, HDL, LDL) and Oxidative test (total oxidant status, total antioxidant capacity, malondialdehyde and catalase) was also performed. In the present research study, use of metformin, *Ocimum sanctum* and combination of metformin and *Ocimum sanctum* significantly reversed the serum levels of AST, ALP and ALT at 21st day of treatment. In alloxan induced diabetic rats total oxidative stress was considerably increased due to pancreatic β cells damage. However, use of metformin, *Ocimum sanctum* and combination of metformin and *Ocimum sanctum* in diabetic rats potentially increased total antioxidant capacity (TAC) and significantly lowered the oxidative stress. For histopathological examinations, tissues sample of pancreas of rats was collected from all experimental groups. The present research study showed that metformin and *Ocimum sanctum* produced better result, combination therapy also showed effectiveness, but it is not much significant as metformin and *Ocimum sanctum* when they used separately. Histopathology of pancreas was performed.

Keyword: Diabetes mellitus, *Ocimum sanctum*, Metformin, Oxidative damage

**P.10. IN-SILICO ANALYSIS AND IDENTIFICATION OF HOT SPOT REGIONS
FOR DIAGNOSIS AND DRUG DESIGNING AGAINST ANO7 GENE IN PROSTATE
CANCER**

*Irtiza Pervez, Syed Hassan Raza, Mina Ellahi, Zulqurnain Ahmed, Aadil Hussain

A-Level, Beaconhouse School System, Kathala, Gujrat

Corresponding author Email: aadil.uog@gmail.com

ANO7 is a potential prostate cancer susceptibility gene. The gene encodes a polytopic membrane protein which may serve as a target in prostate cancer diagnosis and immunotherapy. Nonsynonymous SNPs bring effective modification to the protein structure. In this study, the *missense* SNPs, were analyzed, and their impact on the protein structure was evaluated through the computational tools (SNPS&GO, SIFT, PROVEAN, PANTHER, MutPred, SNAP, MetaSNP, PON-P2, PolyPhen-2, PhD-SNP and I-Mutant2.0). 3D model of the protein was made by using the program I-TASSER. Transcript 201 of ANO7 gene was selected which codes for 999 amino acids protein. Three SNPs (G716C, G716R and G763R), out of seventeen short listed, were declared as the core hot spots of most probable cause of ANO7 to be involved in prostate cancer on the basis of prediction and score, preservation values, p-values and reliability index obtained from the programs. These SNPs can be targeted for diagnose and treatment purpose of this cancer.

P.11. EFFECT OF HIGH-FAT DIET AND CINNAMON ON HORMONAL PROFILE AND ESTROUS CYCLE IN RATS

Aila Tehreem¹, Junaid Ali Khan^{1,*}, Zulfia Hussain¹, Syeda Momna Ishtiaq¹, Haroon Rashid¹,
Kanwal Rehman¹ and Muhammad Shahid²

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Biochemistry,
University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk

Obesity has become an epidemic worldwide and it has deleterious impacts on human health, moreover it casts negative impacts on human fertility characterized by impaired ovulation and polycystic ovary syndrome. *Cinnamomum zeylanicum* is well known for anti-obesity effects and profound anti-hyperlipidemic properties. This study investigated that whether consumption of high fat diet induces polycystic ovaries in experimental animal. For this purpose eighteen young female albino rats were equally grouped into three, group 1 was control and treated with routine diet and group 2 and 3 were experimental groups and were fed with routine diet + 30% margarine and routine diet + 30% margarine + cinnamon extract respectively for 28 days. Blood samples were taken for the analyses of hormonal profile. Vaginal smears were performed on the daily basis to observe the estrous cycle and for histopathological evaluation mice were sacrificed and ovaries were analyzed for any morphological change and cyst formation. The body weight of both treated groups was increased but the high fat diet control group showed a reduction in weight as compared to control and high fat diet treated group. The estrus cycle was severely disrupted in high fat diet group rats and shown prolongation of stages, skipping of stages and some rats have shown acyclicity, but the high fat diet control group has shown prolongation of stages but no stage was skipped or no acyclicity was observed. It was concluded that high fat diet causes polycystic ovary syndrome in animal subjects on a long term basis, whereas cinnamon is beneficial in regulating the hormonal disturbance produced by polycystic ovary syndrome.

Keywords: High fat diet; Cinnamon; Polycystic ovary syndrome; Estrous cycle

**P.12. EFFECT OF ALLOXN-INDUCED DIABETES ON ADRENAL GLANDS
HISTOLOGY AND HORMONAL PROFILE IN ALBINO RATS**

Mehreen Aslam, Junaid Ali Khan*, Zulfia Hussain, Haroon Rashid, Syeda Momna Ishtiaq
and Faqir Muhammad

¹Institute of Pharmacy, Physiology and Pharmacology,
University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk

Diabetes mellitus has now become a drastic trouble worldwide in recent era. In diabetes mellitus, the glucose level of a patient is ultimately increased, as the body can't deliver enough insulin; an essential hormone for transportation of glucose from circulation system to the cell. The present investigation was conducted to assess the effect of diabetes mellitus on the hormonal levels and to determine the antidiabetic impact of zinc oxide nanoparticles and methanolic extract of *Moringa oleifera*. The experiment was performed on thirty albino rats, randomly divided into 6 groups (n=5) i.e., Control (G1), diabetic control (G2), diabetic treated with Glimipride @ 1 mg/kg/day (G3), Diabetic treated with ZnO @ 75 mg/kg/day (G4), diabetic treated with moringa extract @ 250 mg/kg/day (G5) and diabetic treated with both zinc oxide nanoparticles and moringa @ 75 and 250 mg/kg/24 hr (G6). Diabetes was induced with Alloxan infusion intraperitoneally @ 130 mg/kg and rats having blood glucose levels > 250 mg/dl were taken as diabetic. The counter diabetic treatment was given for 28 days. Animals were sacrificed at 28th day of study to collect blood and organ samples for hematological and histopathological analysis. Results show that moringa and zinc oxide nanoparticles treatment significantly ameliorated the diabetic effects on adrenal glands histology and hormonal profile of diabetic rats. It was concluded that the antidiabetic property of moringa and zinc oxide nanoparticles is more when used in combination and these have potential to restore the diabetic altered hematological and adrenal gland cellular structure.

Keywords; Diabetes; Adrenal glands; Hormonal Profile; Moringa; Zinc oxide nanoparticles

**P.13. EVALUATION OF ALLOXAN-INDUCED DIABETIC EFFECTS ON
PITUITARY GLAND AND BLOOD HORMONAL PROFILE IN
MALE ALBINO RATS**

Sadia Hanif¹, Junaid Ali Khan^{1,*}, Zulfia Hussain¹, Haroon Rashid¹, Syeda Momna Ishtiaq¹,
Bilal Aslam¹ and Rao Zahid Abbas²

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Parasitology,
University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk

Diabetes is one of the leading causes of morbidity and mortality in the world. Diabetes mellitus is an endocrine metabolic disorder associated with hormonal imbalance including decreased pituitary hormonal production mainly due to lactotrophs (pituitary) apoptosis. The current study was conducted to determine the antidiabetic activity of plant *Moringa oleifera*, Zinc Oxide nanoparticles and Glimepiride. For this purpose, 30 male albino rats were taken and randomly divided into six groups. The first group was served as control while the 2nd and 3rd were the diabetic control and Glimepiride treated group respectively. Other three groups are treated with *Moringa oleifera*, Zinc Oxide nanoparticles and their combination. After 32 days of study, rats were dissected and blood and organ samples were collected for hematological, biochemical and histopathological analysis. Histopathological investigation showed recovery of damaged pituitary gland cells. Comparisons among these groups were made by student's DMR-test at 5% level of significance. Results show significant ameliorating effects of *Moringa* and Zinc Oxide nanoparticles treatment on alloxan-induced diabetic rats. Hence the experimental study established the fact that *Moringa oleifera*, Zinc Oxide nanoparticles and their combination possessed antidiabetic potential and these agents might be considered as chemically active elements having an antidiabetic activity for new drug development.

Keywords: Diabetes; Pituitary glands; Histopathology; *Moringa*; Zinc oxide nanoparticles

P.14. EVALUATION OF NEPHROTOXIC EFFECTS OF ALLOXAN INDUCED DIABETES ON RAT KIDNEYS

Sehar Bashir¹, Junaid Ali Khan^{1,*}, Zulfia Hussain¹, Haroon Rashid¹, Muzammil Zaman Khan¹ and Aisha Khatoon²

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Pathology, University of Agriculture, Faisalabad, Pakistan.

Diabetes is a metabolic disorder characterized by hyperglycemia and multiple organ dysfunctions due to lack of insulin secretion or its actions. Multiple extrinsic and intrinsic factors play critical role in causation of diabetes. The present study was conducted to evaluate diabetic-induced nephropathy and to study of comparative effects of Zinc Oxide nanoparticles, Moringa and Glimepiride on kidney. To assess the effects of diabetes along with curative effects of zinc oxide nanoparticles, Moringa oleifera alone and in combination, the present study was designed. In this way, a total of thirty male albino rats were randomly divided into six groups. The first group was served as control while the remaining groups were diabetic. Second group was the diabetic control group received only alloxan monohydrate (130mg/kg) body weight. The third group was diabetic and treated with standard antidiabetic drug glimepiride (0.1mg/kg) and fourth group treated with zinc oxide nanoparticles (75mg/kg) per body weight. The fifth group was treated with moringa (250mg/kg) and the sixth group received the combination of zinc oxide nanoparticles and moringa. After 32 days of study, rats were dissected and blood and organ samples were collected on respective days for hematological, biochemical and histopathological analysis. Glimepiride showed the most significant result than all other treatments, zinc oxide nanoparticles and moringa combination showed more significant results as compared to monotherapy in which Zinc oxide nanoparticles showed more significant results than Moringa. It was concluded that combination of zinc oxide nanoparticles and moringa might prove an effective therapy diabetes.

Keywords; Diabetes; Nephropathy; Moringa; Zinc oxide nanoparticles.

**P.15. EVALUATION OF ALLOXAN INDUCED DIABETIC EFFECTS ON
THYROID GLANDS AND BLOOD HORMONAL PROFILE IN
MALE WISTAR RATS**

Tahira Bashir¹, Junaid Ali Khan^{1,*}, Zulfia Hussain¹, Haroon Rashid¹, Muhammad Naeem
Faisal¹ and Rao Zahid Abbas²

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Parasitology,
University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk,

Hyperglycemia, the hallmark of diabetes represents the major cause of metabolic disorders affecting various organs like kidneys, nerves, blood vessels, thyroid glands and eyes. Diabetes alters the level of TSH, T₃ and T₄ and along with retinopathy and nephropathy owing to high sugar levels. Based on this idiosyncratic association of diabetes and thyroid functioning, the present study was designed to evaluate the effects of diabetes on hormonal level and thyroid glands. To evaluate the efficacy of Zinc Oxide nanoparticles, Moringa oleifera leave extract and glimepiride, thirty male wistar rats were taken and divided randomly into six groups. The group one was served as control group while the remaining 5 group were alloxan-induced diabetic groups and were treated with glimepiride, Zinc Oxide nanoparticles, Moringa and their combination. Rats were dissected at 32th day of experiment and blood and organ samples were collected. Effect of diabetes on thyroid functioning has been assessed by hematological, biochemical and Histopathological examination. All these experimental data were statistically analyzed via the use of analysis of variance of one way ANOVA followed by DMR at 5% level of significance. Results show significant ameliorating effects of Moringa and Zinc Oxide nanoparticles treatment on alloxan-induced diabetic rats. Hence the experimental study established the fact that Moringa, Zinc Oxide nanoparticles and their combination possessed antidiabetic potential and these might be considered for their thyroid protecting activity against diabetes for the new drug development.

Keywords; Diabetes; Thyroid glands; Hormonal Profile; Moringa; Zinc oxide nanoparticles

**P.16. EFFECTS OF ALLOXAN-INDUCED HYPERGLYCEMIA ON RAT TESTES
AND REPRODUCTIVE HORMONAL PROFILE**

Umair Rasool¹, Junaid Ali Khan^{1,*}, Zulfia Hussain¹, Haroon Rashid¹, Anas Sarwar Qureshi²
and Rao Zahid Abbas³

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Anatomy,

³Department of Parasitology, University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk

Diabetes is a metabolic syndrome described by chronic increase in blood glucose and multiple organ dysfunctions owing to lack of insulin secretion or its actions on specific receptors. Diabetes affects the normal physiology of reproductive system via actions on hypothalamus, pituitary and gonadal axis. To assess the effects of diabetes along with curative effects of zinc oxide nanoparticles, Moringa oleifera alone and in combination, the present study was designed. In this way, a total of thirty male albino rats were maintained on routine diet and randomly divided into six individuals. The first group was taken as control, while the remaining groups were diabetic. The second group was the diabetic control group received only alloxan monohydrate @ 130mg/kg, the third group was diabetic and treated with standard antidiabetic drug glimepiride @ 0.1mg/kg and fourth group treated with zinc oxide nanoparticles @ 7.5mg/kg body weight. The fifth group was treated with moringa @ 250mg/kg and the sixth group was treated with the combination of zinc oxide nanoparticles and moringa. After 32 days of study, rats were dissected and blood and organ samples were collected for hematological, biochemical and histopathological analysis. Results show that moringa and zinc oxide nanoparticles treatment significantly ameliorated the diabetic effects on testes and hormonal profile of diabetic rats. It was concluded that the antidiabetic property of moringa and zinc oxide nanoparticles is more when used in combination and these have potential to restore the diabetic altered hematological and testes structure.

Keywords; Reproductive hormonal Profile; Testes; Moringa; Zinc oxide nanoparticles

**P.17. EVALUATION OF THE IMPACT OF HIGH SUGAR DIET AND CINNAMON
ON HORMONAL PROFILE AND ESTROUS CYCLE IN RATS**

Muhammad Zubair Anwar¹, Junaid Ali Khan^{1,*}, Anas Sarwar Qureshi², Zulfia Hussain¹,
Haroon Rashid¹ and Farooq Azam¹

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Anatomy,
University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: junaidali.khan@uaf.edu.pk

Obesity is the root cause of a number of health complications that may lead to impaired reproductive system and the hormonal profile. Intake of high sugar diet for a long time period alters the hormonal profile and reproductive cycle mostly in females. Cinnamon has antidiabetic and antiobesity properties. Cinnamon lowers blood sugar level by increasing insulin sensitivity and making insulin more efficient. To assess the effects of cinnamon on high sugar diet-induced obesity, fifteen healthy female albino rats were equally divided into three groups. The first group was served as control and maintained on routine diet, the second group was fed on 30 % sucrose solution whereas the third group was fed on cinnamon aqueous extract in addition to routine diet for 28 days. Blood samples were collected at 0 day and the 28th day of study for the biochemical analysis. Stages of estrous cycle were identified by vaginal lavage analysis on daily basis. Obese female rats were less active, expending more energy per movement, oxidizing more carbohydrates than lean rats. The estrus cycle was severely disrupted in high sugar diet group rats with acyclicity, but the cinnamon treated group has shown slight prolongation of stages but no stage was missed or no acyclicity was observed. It was concluded that high sugar diet causes obesity and polycystic ovary syndrome in animal subjects on a long term basis, whereas cinnamon is beneficial in regulating the hormonal disturbance produced by obesity and polycystic ovary syndrome.

Keywords: Estrous cycle; High fat diet; Hormonal Profile; Cinnamon

P.18. COMPUTATIONAL ANALYSIS OF DIFFERENT PLANT FAMILIES' WRKY DOMAINS FOR THE PREDICTION OF SNPS AND THEIR BINDING CAPACITY

Hamid Manzoor^{1*}, Anum Munir¹, Sumaira Rasul, Ghulam Shabir¹, Sherien Bakht¹,
Tehseen Kanwal¹ and Lubna Rasool²

¹Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, 60800
Multan, Pakistan.

²Department of Chemistry, Sahiwal Subcampus, GC University Faisalabad, Pakistan.

Corresponding author Email: dr.hamid@bzu.edu.pk

In transcription factor families, WRKY is one of the largest families that are extending all over the lineage of green plants. Genes of WRKY family can regulate numerous functions of biological nature by triggering and suppressing many transcriptional processes. In present study, we used different *in silico* tools to examine the synonymous and nonsynonymous Single Nucleotide Polymorphism (SNPs) in WRKY gene family among different plant families *i.e.* Brassicaceae, Poaceae, Fabaceae, Malvaceae, Rosaceae, Apiaceae, Solanaceae, Convolvulaceae, Oleaceae, Cucurbitaceae and Euphorbiaceae. Nucleotide sequences of WRKY gene family were retrieved from NCBI database and BLAST against different plant families to find their homologs. Many SNPs were identified in different members of WRKY gene, containing three important non-synonymous single nucleotide polymorphisms in coding region of *WRKY28* and *WRKY54* in *Raphanus sativus* and *Gossypium hirsutum*, respectively. These SNPs were identified by aligning the WRKY genes with different plant families through Bio Edit. SIFT and Snap2 results revealed that the change of “A” into “T” , “A” to “G” in *RsWRKY28* and change of “A” to “T” in *GhWRKY54* have the highest impact on protein function. These SNPs altered the conserved sequence of WRKYGQK in to WRIYGQK and WRKYGHK in *RsWRKY28* and in *GhWRKY54*, respectively. The functional domain of WRKY protein were modelled by using *AtWRKY1* (PDBID:2AYD) as a template. 3D structures showed that these non-synonymous SNPs lead to change in the structure of protein. These SNPs may have high effect on function and regulation of WRKY genes as our HDock results showed that the proteins having above mentioned SNPs did not bind with DNA.

Key Word: WRKY, Transcriptional regulation, Single Nucleotide Polymorphism, Docking analysis.

P.19. DEVELOPMENT OF TECHNETIUM-99M LABELED EPIRUBICIN FOR INFECTION DIAGNOSTIC RADIOPHARMACEUTICAL

Syed Ali Raza Naqvi^{a*}, Naeem-Ul-Haq Khan^a, Samina Roohi^b,

^aDepartment of Chemistry, Government College University, Faisalabad-38000, Pakistan

^bIsotope Production Division (IPD), Pakistan Institute of Nuclear Science and Technology (PINSTECH), Nilore, Islamabad-Pakistan

Corresponding author Email: dr.aliraza@gcuf.edu.pk

The development of functional imaging is a promising strategy for diagnosis and treatment of infectious and cancerous diseases. In this study epirubicin was developed as a [^{99m}Tc]-labeled radiopharmaceutical for the imaging of multi-drug-resistant *S. aureus* infections. The labeling was carried out using sodium pertechnetate (Na^{99m}TcO₄; ~370 MBq). The other parameters such as amount of ligand, reducing agent (SnCl₂.2H₂O) and pH were optimized. The highest labeling yield ≥96.98% was achieved when 0.3 mg epirubicin, 13 μg SnCl₂.2H₂O, ~370 MBq Na^{99m}TcO₄ were incubated at pH 7 for 15 min in the presence of ascorbic acid at room temperature. Radiochemical purity, stability, charge, glomerular filtration rate, and biodistribution were studied to evaluate the [^{99m}Tc]-epirubicin. Biodistribution investigations showed 13.89 ± 1.56 %ID/gm-organ radiotracer uptake by liver and 7.79 ± 0.38% ID/gm-organ by kidneys at 30 min post-injection which promisingly wash-out at 24 h post-injection. Scintigraphy study showed uptake in *S. aureus* infected tissues in contrast to turpentine oil induced inflamed tissues. Target-to-nontarget ratio (6.7 ± 0.05) was calculated at 1 h post-injection using SPECT gamma camera. The results of this study reveals that the [^{99m}Tc]-epirubicin can be a choice of imaging and monitoring the treatment process of multi-drug resistance *S. aureus* bacterial infections.

**P.20. DNA BARCODING OF PAKISTANI FISH SPECIES USING
MITOCHONDRIAL CYTOCHROME C OXIDASE I (COI), CYTOCHROME B
(CYTB) AND NUCLEAR RHODOPSIN (RHOD) GENES**

Masooma Kynat

Corresponding author Email: masoomakynat14@gmail.com

To overcome fish demand due to increased consumption, management of viable fisheries and controlled inspection of possible replacement fraud across food chain, a more efficient and workable system is needed to increase their growth potential as well as traceability of seafood framework. DNA based identification technologies have been incorporated for lineage information. Moreover, many fish species are sold fraudulently mislabeled due to poor morphological identification especially processed samples lacking morphological characteristics. It is indicated by recent studies that DNA barcoding is a promising technique for fish identification through short specialized sequence of DNA usually 652 base pair region of cytochrome oxidase subunit I gene (COI) of mitochondria. Improved efficacy of DNA barcode was checked by using two genes, mitochondrial cytochrome b (cyt b) and nuclear rhodopsin gene, along with mitochondrial cytochrome oxidase 1 (CO1) in seven species of fish *Wallago attu*, *Oreochromis mossambica*, *Channa marulius*, *Sperita sarwari*, *Notopterus chitala*, *Labeo rohita*, *Rita rita*, of Pakistan. DNA template ranging in concentration of 20ng/μl to 25ng/μl was used. These fish species of Pakistan were genetically analysed by amplification of CO1, Cytochrome b and Rhodopsin genes in the extracted DNA elutes for respective fishes. Six out of 7 species showed quite clear and prominent bands on agarose gel and all of three genes i.e., CO1, Cyt b and rhodopsin were successfully amplified, whereas in *Rita rita* no amplification was observed. PCR Products were purified and sent for sequencing analysis. Sequencing analysis is under process.

P.21. DEVELOPMENT OF SEED PRIMING BASED SHOTGUN STRATEGY FOR IMPROVEMENT OF DROUGHT TOLERANCE IN CHICKPEA

Asima Noreen^{1,2}, Amjad Hameed^{1,2}, Tariq Mahmood Shah^{1,2}

¹Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan

²Department of Biological Sciences, Pakistan Institute of Engineering and Applied Sciences, Nilore, Islamabad

Corresponding author Email: asimanoreen255@gmail.com

Chickpea (*Cicer arietinum* L.) is a cool season legume crop and is grown worldwide as a food source. It is normally grown in arid and semi-arid areas, so drought tolerance is a highly desirable feature. Seed priming is a pre-sowing treatment which leads to a physiological state that enables seed to germinate more efficiently. In recent years, seed priming has been developed as an indispensable method to produce tolerant plants against various stresses. In this study, new seed priming protocols are developed for the improvement in drought tolerance in chickpea. Different seed priming methods i.e; redox priming, hydropriming and on-farm priming were used to find the best treatment. Firstly, screening was done on morphological basis. Then, Biochemical analysis (including total soluble proteins, phenolic contents, total oxidant status, various enzymatic activities, total carotenoids and chlorophyll contents etc.) of primed seeds and resulting seedlings was performed to study biochemical changes induced by priming and effects of priming on drought tolerance of chickpea respectively. Among different seed priming treatments, redox priming for 7 and 12 h were observed as best treatments against drought stress on the basis of morphological and biochemical data analysis. Different levels of enzymatic and non-enzymatic antioxidants provided biochemical basis of drought tolerance mechanisms in chickpea. Best found priming treatment can be recommended for breeders/farmers to attain better drought tolerant crop of chickpea.

**P.22. EFFECT OF PHYLLOSPHERIC BACTERIA ON GROWTH OF
*TRITICUM AESTIVUM L.***

Iffat Fizza and Ambreen Ahmed*

Department of Botany, University of the Punjab, Quaid-e-Azam Campus,
Lahore 54590, Pakistan

Corresponding author Email: ambreenahmed1@hotmail.com

The phyllosphere, which consists of the aerial parts of plants, and therefore primarily, of the set of photosynthetic leaves, is one of the most prevalent microbial habitats on earth. Phyllosphere microbiota are related to original and specific processes at the interface between plants, microorganisms and the atmosphere and play an important role in protecting plants from diseases as well as promoting their growth by various mechanisms. In the current, study twenty one bacterial isolates were selected from the leaf surfaces of *Mangifera indica* and *Alstonia scholaris*. Biochemical constituents of the plant samples were analysed to understand their biochemical composition. Effect of phyllospheric bacteria on plants was recorded by using *Triticum aestivum* and various growth parameters including root length, shoot length, number of leaves and fresh weight were observed and compared with un-inoculated control treatments. The data showed that isolates enhanced the growth of treated wheat plants. Thus, the present work provides an effective technique for plant growth improvement in the agricultural lands.

Keywords: Phyllosphere, bacteria, *Triticum aestivum*

P.23. AN INTEGRATED APPROACH FOR ANALYSIS OF INDIVIDUAL AND COMBINED EFFECTS OF IRRIGATION AND HEAT STRESS IN DESI CHICKPEA

Saima Jameel¹, Amjad Hameed^{1*} and Tariq Mahmud Shah¹

¹Nuclear Institute for Agriculture and Biology (NIAB), Jhang Road, Faisalabad, Pakistan.

Corresponding author Email: saima2864@yahoo.com

Response of desi chickpea against heat stress (H), irrigation stress (I) and a combination of both (I+H) was evaluated using nine genotypes. Irrigation stress was applied 6 days after emergence by maintaining pots at 100% field capacity and control maintained at 50% field capacity. For heat stress, 16 days old seedlings were shifted from net house (day/night temperature 32/10°C) to glasshouse with day/night temperature of 38/17°C. For combined stress, conditions of irrigation stress followed by heat stress were applied. After 30 days of sowing, morphological data and leaf samples for physio-biochemical analysis were collected. Under irrigation stress, D-09027 (increased SOD and TPC, low protease activity) was found tolerant while NIFA-2 (increased total oxidant status, esterase and decreased TPC) as sensitive. Under heat stress D-0913 (increased shoot length, seedling fresh and dry weight, shoot dry and fresh weight, total soluble protein and decreased protease activity) was proved as tolerant and NIFA-2 (decreased TPC, root fresh weight, root dry weight and increased MDA level) as sensitive genotype. Under combined stress, tolerance was observed in D-0913 and CH 24/07 (increased root length and decreased protease and esterase activity) while CMC-211S (increased oxidant status and decreased SOD, protease and TPC) was observed as sensitive genotype.

Keywords: physiological markers, antioxidants, seedling, black seeded, *Cicer arietinum*

P.24. BIO-METHANE POTENTIAL OF LIGNOCELLULOSIC BIOMASSES BY ANAEROBIC DIGESTION AND FIND ITS ECONOMIC FEASIBILITY

Shehbaz Ali^{1,2} *, Tawaf Ali Shah^{1,2}, and Romana Tabassum^{1,2}

1 National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

2 Pakistan Institute of Engineering and Applied Science, Islamabad, Pakistan.

Corresponding author Email: shehbaz205@gmail.com,

Anaerobic digestion is a process to convert organic biomass into bio-methane. Plenty of produced waste in Pakistan is enough to compensate energy thirst of country and have potential to replace costly fossil fuels. The lignocellulosic biomass such as wheat straw, almond shell, sugarcane bagasse, maize straw and corn cob were subjected to bio-methane potential assay after proximate, ultimate and chemical analysis. These chemical fractions provide better understanding about theoretically predicating bio-methane potentials such as neutral detergent fibre, acid detergent fibre, acid detergent lignin, cellulose, hemicellulose, carbohydrates, proteins and elemental analysis. Experimental bio-methane potentials were found, 267.74 (wheat straw), 255.32 (almond shell), 222.23 (corn cob), 247.60 (sugar cane bagasse) and 293.12 ml/g (maize straw) volatile solids and was much less than predicted methane potential. The energy content on dry basis and methane potential has been assessed to find economic feasibility of biomass. The biodegradability and methane potential inversely related to the lignin content of biomass. Bioenergy production from biomass is economically favourable. The volatile fatty acids were produced in the percentage of 53–58% acetic acid, 30–35% butyric acids and 6–13% propionic acid and showed same metabolic pathway and types of bacteria involved in digestion.

P.25. GENOME WIDE ANALYSIS OF CYCLIC NUCLEOTIDE GATED ION CHANNELS AND THEIR ROLES DURING SALT AND DROUGHT STRESS IN ARABIDOPSIS THALIANA

Sadaf Oranab¹, Bushra Munir¹, Zulqurnain Khan², Sultan Habibullah Khan², Aftab Ahmad^{2,3*}

Abdul Ghaffar^{1*}

¹Department of Biochemistry, Government College University, Faisalabad, Pakistan

²US-Pakistan Center for Advanced Studies in Agriculture and Food Security, University of Agriculture, Faisalabad, Pakistan

³Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: aftab.ahmad@uaf.edu.pk

Cyclic nucleotide gated ion channels (CNGCs) regulate important biological processes in plants as well as animals. In this study, all 20 members of CNGC family of Arabidopsis thaliana (*A. thaliana*) were analyzed for their phylogenetic relationship, location on chromosomes, domains and motif analysis, protein analysis and their expression under abiotic stresses. In addition, comparative phylogenetic relationship of CNGCs was analyzed between *A. thaliana*, barley and rice to evaluate their evolutionary linkage. Cyclic nucleotide gated ion channels (CNGCs) in plants, animals and prokaryotes have very important role in signaling and development. Plants face abiotic stresses which greatly influence the production and yield of crops. CNGCs are differentially expressed under these stresses and minimize the effects of fluctuations. They have individually different role in signaling, development, biotic and abiotic stress. In this study, we retrieved twenty CNGCs of *A. thaliana* from the online database and analyzed their structure and properties by using bioinformatics tools. It was analyzed that maximum number of CNGCs are located on chromosome 2. CNGCs 4, 5, 6 and 9 have long untranslated introns, while CNGCs 7, 8, 13 and 16 have fully translated exons and introns. Maximum number of ESTs were present in CNGC 2 while CNGC 6 had no ESTs. In addition, CNGC 20 had highest molecular weight as well as number of amino acid residues, while CNGC 11 had lowest molecular weight and amino acid residues. Moreover, domain and motif analysis showed that ion transport domain was common in all CNGCs of *A. thaliana*, while ion transport 2 motif was only present in CNGC6 and CNGC9. The presence of common motif 24 on HvCNGC19, OsCNGC4, OsCNGC11 and AtCNGC19 depicted that, they might be expressed similarly as AtCNGC19 under salt stress. The phylogenetic relationship of abiotic stress related CNGC19 in *A. thaliana* with Barley and Rice depicted that HvCNGC 19, OsCNGC4 and OsCNGC11 are closely related each other. CNGCs expression under abiotic stresses showed

that CNGC 19 and CNGC20 of group IVa were highly expressed in roots under salt stress after 6 hours and expression was low after 12 hours. The study provides new insights of AtCNGCs and will be helpful for functional genomics of CNGCs in plants as well as characterization of CNGCs for their roles during biotic and abiotic stress.

Keywords: Arabidopsis thaliana, Cyclic nucleotide gated ion channels, Phylogenetic relationship, salt and drought, motif analysis

**P.26. SYNTHESIS OF MERCAPTAN-TERMINATED POLYTHIOURETHANES:
VERSATILE CURING AGENTS FOR EPOXY RESINS**

Najum Abbas¹, Ayesha Naseer¹, Sadia Nazir², Javed Iqbal¹, Sadia Noor¹, Tehreem Naz¹,
Muhammad Abid Rashid^{1*}

¹Department of Chemistry, University of Agriculture, Faisalabad 38040, Pakistan

²Department of Chemistry, Government, College Women University, Faisalabad, Pakistan

Corresponding author Email: marashid@uaf.edu.pk

Epoxy resins are reactive intermediates used to produce a versatile thermosetting polymers. Curing agents are of paramount interest and extensively used to build up hard, infusible thermoset networks. New mercaptan terminated polythiourethane have been synthesized by condensation of diisocyanates with dimercaptans. Reaction of toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI) with dioxo-1,8-octane-dithiol (DOT) and tetra(3-mercaptopropionate) pentaerythritol (PETMP) afforded new elegant desired curing agents . The structures of synthesized products were confirmed by FT-IR spectroscopy and physical parameters like viscosity and refractive index were also determined. Usage of 20Phr (Per hundred resin) curing agents cured the epoxy resin in 3-7 hours as indicated by FT-IR. The mechanical properties like tensile strength, impact strength, refractive index and viscosity of cured epoxy resins were characterized by ISO methods. The prepared polythiourethane hardeners have an acceptable odor and give a perfectly homogeneous system with epoxy resins.

Keywords: Polythiourethane, Epoxy resin, FT-IR, Mechanical properties,

**P.27. EVALUATION OF INTEGRONS AMONG CEPHALOSPORIN RESISTANT
ESCHERICHIA COLI AND *KLEBSIELLA PNEUMONIAE* ISOLATED
FROM BURN PATIENTS**

Hafsa Yousaf and Muhammad Asif

Institute of Molecular Biology and Biotechnology

University of Lahore, Pakistan

Corresponding author Email: hafsasandhu72@gmail.com

Integron system is a dynamic force in the evolution of multidrug resistance (MDR) and it helps bacteria to acquire novel combinations of and CTX-M type β lactamases increasingly becoming predominate in recent years in *Escherichia coli* and *Klebsiella pneumoniae*. Integrons and extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria caused Nosocomial infections make difficult treatment and limit therapy choices. *Escherichia coli* and *K. pneumoniae* isolates with reduced vulnerability to cephalosporins were collected from different hospital units and patient pus. We determined antibiotic susceptibility and detect the genes encoding CTX-M enzyme and 1, 2 and integrons among the *Escherichia coli* and *Klebsiella pneumoniae* isolated from pus of burn wounds samples. 35 clinical samples of *Escherichia coli* (n=7) and *Klebsiella pneumoniae* (n=28) collected from different hospitals in Lahore. The susceptibility of isolate to 7 antibiotics disks was determined by using disk diffusion method. The presence of CTX-M gene and class 1, 2 and 3 integrons was investigated by PCR. CTX-M in *K. pneumoniae* and *Escherichia coli* classes were identified in 53% and (57.1%) respectively. *K. pneumoniae* 15 (53.5%) were carrying integron class 1, 4 (14.2%) with integron class 2 and both the integrons 1 and 2 classes were present in 17% of the samples. Integrons class 1 was identified in 5(71.4%) of *E. coli* isolates, 2 (28%) were identified with integrons class 1 and 2.

P.28. BIOCHEMICAL ANALYSIS AND CHARACTERIZATION OF MUCUS FROM THE SKIN OF COMMON CARP

Sehrish Hanif¹, Hina Fatima², Muhammad Shahid², Urooj Khalid¹.

1-Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad.

2-Department of Biochemistry, University of Agriculture, Faisalabad.

The water environment for fish is very competitive due to the presence of large number of microorganisms, so the fish mucus provides protection against these pathogens and microorganisms. Against these pathogens, fish mucus provides first line of defense. The emerging species of fish, *Cyprinus carpio* have extremely practical importance in the field of aquaculture in Pakistan and other country of Asian subcontinents. The main objective of this research was to designate the fish mucus in respect to its biochemical analysis and characterization. This involves various methods such as agar well diffusion method for assessment of antimicrobial activity. For determining the fish mucus antioxidant activities such as total phenolic contents, total flavonoid contents, DPPH and reducing power were done. The cytotoxicity of fish mucus was checked by the haemolytic and thrombolytic activities. CAT, POD, SOD and protein estimation were also done for biochemical analysis of fish mucus. Moreover, various other techniques such as UV-visible spectra, FTIR were used for the characterization of fish mucus. Mean and standard deviations were used for the analysis of results.

P.29. EFFECT OF WATERBORNE CADMIUM EXPOSURE ON GLUTATHIONE S TRANSFERASE (GST) IN WALLAGO ATTU LIVER

Yasmeen Batool¹, Muhammad Anjum Zia², Sajid Abdullah¹, Zaheer Ahmed² Hadia Naseem²

¹Limnology –Fish Nutrition Laboratory, Department of Zoology, Wildlife and Fisheries,

²Enzyme Biotechnology Laboratory (EBL), Department of Biochemistry,

University of Agriculture, Faisalabad, Punjab 38040, Pakistan.

Corresponding author Email: mbczaheer@hotmail.com

The widespread industrialization has resulted in the exposure of their toxic residues in various environmental segments. Heavy metal contamination of surface waters has been well documented worldwide and constitutes a major issue that gives rise to concerns at local, regional, national and global scales. Heavy metals have the ability to generate reactive oxygen species (ROS) that would cause oxidative stress. Antioxidants enzymes can be used as a good biomarker of oxidative stress. Therefore, in present research work was designed to evaluate the Glutathione S-transferase activity in liver of Wallago attu exposed to 96-hr LC₅₀ concentration of (32.96mgL⁻¹) at sub-lethal concentrations for fourteen days. Sampling was done after 7 days. After each sampling fish were sacrificed and required organ viz. liver was removed to estimate the activity of glutathione S transferase. Results showed that the exposure of waterborne cadmium increased the GST activity in liver of fish as compared to control. It was also concluded that GST activity increased as the exposure period increase. The maximum GST activity was noted after 14 days of the exposure period.

Keywords: Glutathione S transferase (GST), Cadmium, Toxicity. Wallago attu, ROS.

**P.30. PRODUCTION AND PURIFICATION OF STREPTOKINASE FROM
BACTERIAL STRAIN *STREPTOCOCCUS DYS GALACTIAE* SUBSP.**

DYS GALACTIAE

Ghulam Akbar¹, Muhammad Anjum Zia¹, Khalil-ur-Rehman¹, Muhammad Shahid¹,

Khalid Nadeem¹, Muhammad Hayat¹ and Muhammad Bilal¹

¹Enzyme Biotechnology Laboratory, Department of Biochemistry,

University of Agriculture, Faisalabad

Corresponding author Email: ghulamakbardgk@gmail.com

There are many B-hemolytic *Streptococci* which produce streptokinase (SK). It's a protease enzyme with molecular weight 47kDa and comprising 414 amino acids. It's function is to dissolve blood clots formed in the blood vessels and used to cure cardiovascular diseases (CVD) which are leading cause of death along the World. In this research study streptokinase was produced from *Streptococcus dysgalactiae* in the fermentation medium. Well grown *streptococcus dysgalactiae* colonies were screened out by applying colonies restrictor, triton x-100. Different fermentation parameters such as pH, fermentation time, glucose concentration, yeast extract and substrate concentrations were optimized by response surface method. Analysis of data was made by following the ANOVA. Purification process of Streptokinase was performed by ammonium sulfate precipitation, ion exchange chromatography and gel filtration. Purified streptokinase expressed enzyme activity 252.0U/mL, specific activity 93.54U/mg and was obtained with 78.37% recovery.

Key words: Streptococcus, Streptokinase, Optimization, Production and purification

P.31. KNOWLEDGE ASSESSMENT OF OBESITY, ITS MANAGEMENT AND LIFESTYLE OF PAKISTANI POPULATION; A SURVEY-BASED STUDY

Muhammad Ahsan ul Haq¹, Muhammad Numan^{1*}, Maria Arshad¹, Adnan Kabeer¹

Department of Biochemistry, Government College University, Faisalabad, Pakistan.

Corresponding author Email: noooomi143@gmail.com

Healthy food and lifestyle play a vital role in controlling our health and body mass index (BMI). This study was designed to analyze the knowledge of people about obesity, its management, healthy food and lifestyle living in different cities of Punjab. A random cross-sectional study was executed by recruiting 536 study participants. The mean age was 22.22 ± 7.98 , and 128 (23.88%) males and 408 (76.11%) were females. Out of 536 objects 114 (21.26%) were under-weight, 309 (57.64%) were normal, 45 (8.39%) were over-weight and 68 (12.68%) were obese. Results showed that 69.30% of under-weight study participants had poor, 27.19% average and 3.51% had good knowledge score, while 64.72% of normal weight study participants had poor, 33.33% average and 1.94% had good knowledge score. 48.89% of over-weight people had poor, 44.44 % average and only 6.67% had good knowledge score while 50% of obese had poor, 47.06 % average and 2.96% had good knowledge score about obesity and life style. 15.67% preferred fast food over home-made food while 47.94% people don't follow healthy eating plan and 62.87% of total participants don't exercise regularly. This study showed very low awareness about obesity, life style including their diet and physical activities. Unhealthy diet and sedentary life style are major risks factors for obesity. So, there is immediate need to organize public sector seminars regarding obesity, its management and life style of people in Pakistani population.

Keywords: Obesity, Knowledge assessment, Fast food, Obesity complications, Obesity management.

P.32. NANOTECHNOLOGY: A MODERN TOOL TO CONTROL MOSQUITOES AND MOSQUITO-BORNE DISEASE

Muhammad Abdullah Malik*¹, Muhammad Sohail Sajid ^{1,2}, Muhamamd Imran¹, Kashif Hussain³, Syed Hussain Ahmad Shah⁴, Mahnoor Akbar⁴, Tean Zaheer¹

¹Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

²Center for Advanced Studies in Agriculture and Food Security (CAS-AFS) University of Agriculture, Faisalabad, Pakistan

^{3,4}Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan

Corresponding author: Email: abdullahmalik42@gmail.com

Vector control is an essential requirement in control of epidemic diseases such as malaria, filariasis, dengue that are transmitted by different species of mosquitoes. Due to development of resistance against insecticides and their detrimental effects on public health, modern science discovers new methods to control mosquitoes and mosquito-borne diseases. These methods are efficient, economical, eco-friendly and do not harm the non-specific targeted organisms. One of the modern control methods developed to control this deadly creature is synthesis of nanoparticles from naturally occurring herbs. These nanoparticles include silver, gold, copper and silicon etc. The development of these nanoparticles helps to formulate new insecticides and insect repellent with minimized or no resistance. The structure of these nanoparticles can be identified using modern technologies i.e. UV-VIS spectroscopy, X-ray diffraction, Scanning and Transmission Electron Microscopy and many others. There is a very wider field in which nano-biotechnologies can be applied including; diagnosis of diseases, drug delivery, tissue engineering, gene delivery, artificial implants, pest management, imaging and sensing etc. the biosynthesis of nanoparticles from green plants which is also known as green synthesis is very advantageous as compared to chemical and physical methods of preparation because preparation from herbs does not require any toxic chemical, less time consuming and very minute use of pressure, energy and temperature is there. Particularly, increasing number of plants and fungi is proving effective in extracellular production of gold and silver nanoparticles which are very effective against viruses. However, this aspect of nanotechnology has not been explored effectively in controlling mosquitoes or other vectors. This paper will represent a review about the production of nanoparticles from herbs and their potential in controlling mosquitoes and mosquito-borne diseases.

Key words: Nanotechnology, silver, gold, mosquitoes, dengue, mosquito-borne diseases

P.34. BIOCHEMICAL AND NON-BIOCHEMICAL FACTORS AFFECTING COLOR AND SHELF LIFE OF TOMATO KETCHUP

Tahreem Arshad Butt

Institute of Biochemistry and Biotechnology, Punjab University

In recent study, tomato ketchup a product of tomatoes (*Lycopersicum esculentum*), and factors affecting its color and shelf life were studied. Tomato ketchup is a sweet tangy sauce that contains carotenoids, lycopene, vitamin A, vitamin B, vitamin C, potassium, magnesium, and iron. Tomato ketchup prevents is proved healthful in preventing night blindness, cardiovascular disorders, cancer and lowering blood cholesterol level. Factors causing browning of tomato ketchup were studied. Lycopene isomerized from trans to cis form by non-enzymatic oxidation. Lycopene degradation leads to browning of ketchup. Biochemical factors such as lycopene, preservatives, food acids, polyphenolic compounds causing browning of ketchup were studied. Non-biochemical factors like temperature, PH, vacuum packaging, packaging material, heat treatment, instant cooling and storage conditions effected the color and shelf life of tomato ketchup. In this study, we had taken different tomato ketchup samples for detection of nutritional composition of ketchup. Organoleptic and proximate tests were performed. Effect of packaging material and temperature alone and in combination with 0.5% ascorbic acid, 0.2% citric acid (CA) and instant chilling on shelf life of tomato ketchup were determined. Citric acid and ascorbic acid prevented darkening of tomato ketchup. Packaging material such as aluminum pouches are better in preventing darkening of ketchup then polythene bag. Non-enzymatic browning was prevented by vacuum packaging.

P.35. SYNTHESIS OF LOUREIRIN B LsOADED NANOLIPOSOMES FOR PHARMACOKINETICS IN RAT PLASMA

Aysha Zafar, Shaheen Qasim, Kinza Mehmood, Huma Gulzar,
Mah Rukh Altaf and Murtaza Hasan

Department of Biochemistry & Biotechnology, The Islamia University of
Bahawalpur, Bahawalpur 63100, Pakistan

Leureirin B (LB) is a most radioactive compound of dragon's blood but its poor pharmacokinetics restrict its clinical applications. Here, we have synthesized phospholipids and cholesterol based nanoliposomes (NLs) using thin film evaporation technique as nanocarrier for LB to overcome the associated clinical issues. The as-synthesized NLs show zeta potential of -51.2 mV and an average size of 84 nm which is highly favorable for high entrapment efficiency for LB that reaches to 74.5%. In-vitro flow cytometry results reveal that LB-NLs recover the radiation injury in viable cell from 79.4% to 89.9%, early apoptosis from 3.5% to 0.2%, necrosis from 14.8% to 9.8% and late apoptosis from 2.3% to 0.0%. In-vivo assay, LB-NLs successfully improved the pharmacokinetic parameters such as maximum concentration of LB-NLs formulation, elimination rate half-life, area under curve, and plasma clearance are 3.247 ± 0.631 ng/mL, 14.765 ± 10.780 min, 2.957 ± 0.201 and 0.132 ± 0.901 ng/mL, respectively. Thus, we believe that designing of such unique LB-NLs composite not only improved the anti-radiation characteristics of LB but also enhanced its solubility that increased its ability for the drug-delivery.

KEYWORDS: Leureirin-B, nanoliposomes, cell cycle, zeta potential, pharmacokinetics

P.36. BIOCHEMICAL COMPOSITION AND TOXICOLOGICAL SCREENING OF GUTKA

Saiqa Jamil¹, Muhammad Shahid², Hina Fatima²

1 Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

2 Department of Biochemistry, University of Agriculture, Faisalabad.

Gutka is a form of smokeless tobacco product and widely used drug throughout the world. It is composed of about 4,200 chemicals and carcinogenic substances such as nickel, lead and chromium, which make it more harmful than any other form of tobacco. It is also contain different toxicological properties and considered a carcinogenic and mutagenic agent. Present research project has been designed to evaluate the comparative biochemical composition and toxicological screening of gutka of two different companies. Both methanolic and ethanolic extracts of gutka was prepared. Cytotoxicity of gutka extracts was checked through hemolytic assay against Human Red blood cells and against oxidative DNA damage induced by hydrogen peroxide. Mutagenecity and non-mutagenecity of gutka was performed against TA-98 and TA-100. All extracts (methanolic and ethanolic) were showed significant mutagenicity and cytotoxic potential. The chemical profiling of gutka extract was performed through UV-visible spectra, FT IR spectra, and atomic absorption spectrophotometry. In UV-visible spectrum highest peak was obtained at 200nm and 500nm in brand 1 and 200nm and 450nm in brand 2.. In FT-IR nitro, free alcohol, alkane functional groups were detected. Through Atomic Absorption Spectroscopy different toxic metals (Be, Cr, Co, Ni, As, Cd, Ba, Pb) were detected in smokeless tobacco gutka. All results obtained were analyzed through Mean $\pm$ S. E.

Keywords: Gutka, Cytotoxicity, Mutagenicity, Atomic Absorption Spectroscopy, Fourier transformed infrared spectroscopy, UV-Visible spectroscopy

P.37. BIOLOGICAL ACTIVITIES OF *STEVIA REBAUDIANA* LEAVES IN VARIOUS ORGANIC SOLVENTS

Muhammad Ghous, Fatma Hussain*, Muhammad Shahid

Corresponding author Email: fatmauaf@yahoo.com

Objective: To evaluate the anti-oxidant, anti-diabetic, anti-microbial and cytotoxic potentials of *Stevia rebaudiana* leaves. **Methods:** The extracts were then dissolved in distilled water and fractionation was done using different polarity based solvents such as *n*-hexane, *n*-butanol, ethanol, methanol, ethyl acetate and chloroform in separating funnel. The antidiabetic activity of the fractions were measured along with other biochemical attributes including antioxidant, antibacterial, antifungal, cytotoxic, hemolytic, thrombolytic and compared with that of the control values. **Results:** The fractions of *Stevia* leaves showed significant in antioxidant and antidiabetic activity. The chloroform fraction showed maximum antibacterial and *n*-butanol fraction showed maximum antifungal activity. The ethanol, *n*-butanol and aqueous extract/fractions damaged the DNA. The *n*-butanol exhibited the maximum hemolytic and *n*-hexane showed highest thrombolytic activity. **Conclusion:** The study findings have shown that the fractions of *Stevia rebaudiana* leaves exhibited the significant antioxidant and antidiabetic effects. Therefore, we suggest the use of *Stevia rebaudiana* leaves as a natural remedy in treating and regulating diabetes.

Keywords: *Stevia rebaudiana*, diabetes, antioxidants, antimicrobial and cytotoxic activity.

P.38. MEDICINAL ASPECTS OF ZINGIBER OFFICINALE ROSCOE: A REVIEW

Izah Laraib

Department of Horticulture PMAS Arid Agriculture University Rawalpindi, Punjab, Pakistan

Ginger (*Zingiber Officinale*) is a medicinal plant native to Asia. As an anti-inflammatory agent it is globally known for having medicinal properties. For over 25 centuries it has also been used as traditional Chinese and Indian medicine. The purpose of this research is to highlight the recent studies on medicinal value of Ginger. Summarized data is used in this paper and data is collected from different articles, papers and books. In the early 1970s it has been repeatedly confirmed that ginger has inhibitory effects on prostaglandin biosynthesis. The discovery also declared ginger as a herbal medicinal product having pharmacological properties. It is considered as an antioxidant, chemopreventive, hypoglycemic and antiplatelet. It is also used as cholesterol lowering and blood pressure lowering agent. It is also reported that it is used in prevention of nausea either caused due to cancer treatment, HIV, AIDS treatment and nausea after surgery. It is also prescribed in the treatment of Rheumatoid Arthritis. Recent studies show that Ginger also has number of uses in dermatology. Its fresh juice can be used to treat burns on skin. Its extracts use in prevention of insect bite and oil use on skin to cure pain. An experiment proved that ginger is also used in the treatment of gouty dyspepsia, during that experiment the roots of ginger were powdered in a mortar and a teaspoon of that powder was added into boiling milk and that drink proved effective to this disease. Its powder from one to twenty grains can be added in hot and cold water to make syrup and one to two teaspoons of this syrup proved effective in the treatment of diarrhea and weekly chronic bronchitis.

P.39. BIOCHEMICAL ANALYSIS AND CHARACTERIZATION OF MUCUS FROM THE SKIN OF LABEO ROHITA

Urooj Khalid¹, Hina Fatima², Muhammad Shahid², Anam Atta¹, Shagufta Ramzan¹
and Sehrish Hanif¹.

¹ Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

² Department of Biochemistry, University of Agriculture, Faisalabad.

Fishes are live in the environment that is rich in pathogens so, the mucus of the fish showed antimicrobial properties to inhibit these pathogens. The epidermal mucus of the fish provides first line of defence against pathogens. Present study had been designed to characterize the antibacterial activity and biochemical composition of the fish skin mucus against different bacterial strains. Fish specie *Labeo rohita* had been used for studied the analysis of their mucus sample and mucus for the activities of antibacterial against the many Gram positive or Gram negative bacteria. Overall results of activities showed that crude mucus extract exhibited higher activities than that of saline mucus extract. Antimicrobial potential of fish mucus was evaluated through well diffusion method. Crude mucus extracts was showed slightly higher activity of antibacterial against Gram negative (*Escherichia coli*, *Pasterulla multocida*) and Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*,) bacteria than the saline mucus extract of *Labeo rohita*. Samples were tested for their haemolytic and thrombolytic activities. The fish mucus activities of antioxidants were checked by DPPH, reducing power, total phenolic contents (TPC), total flavonoid content (TFC) and for biochemical analysis catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and protein estimation was also done. Similarly, the advanced techniques of fourier infrared spectroscopy (FTIR) and UV spectra had been used for the characterization of the fish mucus. FTIR results in fish mucus showed the presence of aliphatic primary amines (N-H) and alkenes as a functional group (C=C) at different peaks of spectrum. The results obtain were analyzed through mean and standard deviations.

Keywords: Fish mucus, Bichemical analysis, antimicrobial potential, UV-Visible spectroscopy, FTIR

P.40. GREEN NANOBIPARTICLES: ALTERNATIVE CONTROL STRATEGY AGAINST TICKS

Tejan Zaheer^{1*}, Muhammad Sohail Sajid^{1, 2}, Mahvish Maqbool¹
and Muhammad Abdullah Malik¹

¹Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

²Center for Advanced Studies in Agriculture and Food Security (CAS-AFS)

University of Agriculture, Faisalabad, Pakistan

Corresponding Author Email: teanzaheer942@gmail.com

Ticks (Acari: Ixodidae) are obligate hematophagous ecto-parasites of domestic and wild animals causing economic losses through direct (e.g. bite, damaged hides, anemia etc.) and indirect (tick-borne pathogens) means. The development of eco-friendly acaricides gained currency in view of the development of resistance. Green nanoparticles (microscopic particles derived from plants having at least one dimension less than 100 nm), are also successful alternates for effective control of acari through intervention in the metabolic pathways. The green synthesis of nanoparticles is a single step procedure characterized by bio-reduction which yields multifunctional metabolites. The green synthesis of various nanoparticles e.g. Argentum, Aurum, Copper, Cobalt, Lead, Silicon, Zinc and many more have been done so far. The nanobiparticles primarily cause oxidative stress and alter metabolism of ticks at cellular level by disrupting deoxyribonucleic acid during the replication and cell division. Green nanobiparticles may also reduce acetylcholinesterase activity, or may inhibit CYP450 isoenzymes. Some nanoparticles can act as trypsin inhibitors and disrupt development and reproduction. Moreover, the green nanoparticle production is a cost effective procedure which requires low maintenance. Reports have shown that green nanotechnology has caused up to 100% mortality when used against ticks. The use of green nanobiotechnology against hematophagous parasites of economic importance may provide alternate control strategies as this technique has shown commendable results against various species of ticks, mosquitoes, lice and mite in lab trials.

Keywords: Nano-biparticles, control, ticks.

P.41. BIOCOMPATIBLE ONE POT GREEN SYNTHESIS OF MANGANESE DIOXIDE NANOCAPSULES USING FLOWER EXTRACT OF CRYOTA MITIS

¹Muniba Rehmat*, Haq Nawaz Bhatti^{1*}, Sufyan Rahmat¹, Waheed S. Khan², Asma Rehman².

¹Department of Chemistry, University of Agriculture, Faisalabad-Pakistan

²Nanotechnology Group, NIBGE, Jhang Road Faisalabad-Pakistan

Corresponding author Email: rahmat.muniba@gmail.com; hnbhatti2005@yahoo.com

Innovative green technologies for synthesis of metal/metal oxide nanoparticles getting tremendous attention due to their interesting and potential applications. Traditionally used chemical and physical methods for nanoparticle synthesis needs toxic organic solvents, hazardous byproducts and high energy consumption and thus pose a major threats to human health. In this context an environmentally benign one pot approach have been developed during present study for fabrication of manganese dioxide nanoparticles using plant extract as sole reducing and stabilizing agents. Flower extract of medicinal plant *Cryota mitis* (Fishtail Palm) was used as an efficient reducing agent for synthesis of stable manganese oxide nanostructures. Completion of reduction has observed by color variation from yellow to dark brown and was further confirmed by characterization techniques such as X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) showed Nanocapsule morphology, Fourier Transform-Near Infrared Spectroscopy (FT-NIR) showed a characteristic peak for Mn₂O₃ bond stretching at 684.07nm. Zeta potential of -3.04 mv was noticed that favors its electrical stability. Such types of MnO₂ nanomaterials will be expected more potent for antimicrobial activity, photo catalytic dye degradation and sensing compared with other metal and metal oxide nanostructures.

Keywords: Nanoparticles; Green Synthesis; Nanocapsule; XRD

P.42. CHEMOTHERAPY VS ENZYME THERAPY: EFFECTIVE TOOLS FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Hadia Naseem¹ Muhammad Anjum Zia¹, Zaheer Ahmed¹, Yasmeen Batool¹,

¹Enzyme Biotechnology Laboratory (EBL), Department of Biochemistry,

University of Agriculture, Faisalabad, Punjab 38040, Pakistan.

Corresponding author Email: mbczaheer@hotmail.com

Cancer is a renowned production of neoplastic cells. In cancer, bone marrow and other blood-forming organs produce immature cells that start in the bone marrow and enter the blood and inhibit the production of normal cells and cause anemia. Several oncogenes, including the *p53*, *c-fms* and *Ras genes*, can be activated by point mutations that change the amino acid sequence in the critical portion of protein. L-asparaginase is an enzyme which, by hydrolysis, produces aspartic acid and ammonia. It is used as medicine and in the food industry. It is also used as an important chemotherapeutic agent for the treatment of a variety of lymphoproliferative syndromes and acute lymphoblastic leukemia (ALL) L-asparaginase Reduce the level of L-asparagine in plasma and results of inhibition of RNA and DNA synthesis. Because of the unique mechanism of action against cancer, L-asparaginase has been introduced in multi-drug chemotherapy in children and adults in acute lymphoblastic leukemia, it has donated the important development in therapy results and helps achieve reform sullener approximately 90% of patients.

Keywords: Acute Lymphoblastic Leukemia (ALL), point mutation, L-asparaginase, Chemotherapy & Enzyme Therapy.

P.43. BIOCHEMICAL CHARACTERIZATION OF ERUCA SATIVA AND DETERMINATION OF ITS ANTIOXIDANT POTENTIAL

Hajira Amjad, M. Altaf Hussain and Raja Tahir Mahmood

Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 (AJK) Pakistan

Present research was conducted on the basis of biochemical characterization. Biochemical characterization of taramira performed through protein banding profile using Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS-PAGE). Cluster analysis and principal component analysis (PCA) reflected significant difference in all 12 lines of taramira. Antioxidants activity was measured by using DPPH radical scavenging method. The variation in protein was analyzed by using SDS-PAGE to scrutinize the genetic miscellany of Eruca sativa. Twelve lines of taramira were obtained for investigation of genetic diversity for total seed proteins using SDS-PAGE. The resolving gel was prepared by mixing 1.3ml (1.5M Tris Hcl pH8.8), 1.9ml (distilled water), 1.7mL (30 % acrylamide), 50 uL (APS 10 %) and 2ul TEMED was added at the end. The stacking gel was prepared by mixing 0.13ml (1M TrisHcl Ph 6.8), 0.68ml (distilled water), 0.17 ml (30 % acrylamide), 10 ul (SDS 10 %) and 1ul (TEMED) at the end. By calculating the protein bands on the gel, data matrix was prepared. In the data matrix the presence of each band was noted as 1, while the absence of every band was recorded as 0. The data organization consisted of a binary data (0\1), which was designed to evaluate the genetic diversity of twelve lines of taramira by using Hierarchical cluster analysis with the utilization of computer analysis. On the basis of present research work it is concluded that Eruca sativa lines have variation in biochemical constituents. The protein bands pattern reveal a rather low genetic diversity among the selected twelve lines signifying a higher extent of genetic similarity among them.

Keywords: Eruca sativa, Antioxidant, biochemical characterization, DPPH

P.44. MORPHOLOGICAL AND BIOCHEMICAL RESPONSE OF WILD AND CULTIVATED GENOTYPES OF CARROT (*DAUCUS CAROTA* L.) UNDER *IN VITRO* SALT STRESS CONDITIONS

Hina Manzoor, Aamir Ali, Naima Huma Naveed

Department of Botany, University of Sargodha, Pakistan

Corresponding author Email: manzoorhina45@yahoo.com

Due to exponential population growth and increasing anthropogenic pressures, food security is in imminent danger which requires a practical approach to sustainable agriculture in order to meet the ever increasing global food demands. Soil salinity is a contemporary global issue which is faced by agricultural sector now days as it impedes the crop yield and its survival which leads to decreased market value. Carrot is a major agricultural crop which is beneficial for human health but has substantial economic value. Therefore, the present *in vitro* study focused on morphological and biochemical responses of local wild (*GB*) and local cultivated (*T-29*) genotypes of *Daucus carota* under salt stress conditions. Seeds were germinated on MS basal medium over a range of different salt concentrations (0, 50mM, 100mM, 150mM, 200mM, 250mM, 300mM, 350mM, 400mM and 450mM NaCl) with germination and growth observed only up to 150mM NaCl in both the genotypes. Overall, a decreasing trend in morphological attributes and an increasing trend in biochemical attributes were noticed. However, a mixed response of both carrot genotypes to different yield contributing as well as biochemical parameters was recorded which suggested a significant genotype effect. Local wild (*GB*) genotype was higher in its fresh weight, number of leaves, number of roots, total soluble amino acid contents, non-reducing sugars and polyphenol oxidase activity. Whereas, local cultivated (*T-29*) genotype had higher germination percentage, shoot length, root length, total soluble protein contents, total soluble sugars, reducing sugars and peroxidase activity. Among different NaCl concentrations used in the present study, 150mM was found to be highly inhibitory for carrot germination, growth and survival. Furthermore, the present investigation generated some useful information on optimization of NaCl treatments used and standardization of *in vitro* screening protocol against salt stress.

Keywords: carrot (*Daucus carota* L.), *in vitro*, salt stress, germination, growth, survival

**P.45. EXTRACTION OF BIOACTIVE COMPOUND FROM GRAPE FRUIT PEEL
USING DEEP EUTECTIC SOLVENT**

Hira Ishfaq¹, Muhammad Zahid*¹, Zubera Naseem¹, Muhammad Asif Hanif¹, Muhammad Shahid², Asim Mansha³

¹ Department of Chemistry, ² Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

³ Department of Chemistry, Government College University Faisalabad, Pakistan

Corresponding author Email: rmzahid@uaf.edu.pk

Grape fruits (*Citrus paradisi* Macfad) are widely used in food industries for manufacturing citrus-based drinks or fresh juices and rich in phytochemicals, which are known to have health maintaining properties. The deep eutectic solvents are analogous to ionic liquids. These are safer compared to traditional organic solvents due to environment friendly nature such as non-toxicity, biodegradability, negligible vapor pressure, etc. The deep eutectic solvent was prepared by combination of choline chloride (ChCl) and ethylene glycol (EG). The characterization of synthesized solvent was done by (Fourier Transform Infrared Spectroscopy (FT-IR)). The ultrasound-assisted, microwave-assisted and maceration were used for the extraction of bioactive compounds from grape fruit peel. The quantification and characterization of extracted compounds was done by high performance liquid chromatography (HPLC). Different parameters like time, temperature, solid to solvent ratio, were optimized for the maximum extraction efficiency. The total phenolic and flavonoids contents and their antioxidant activities were assessed by established biological protocols. The optimization and interaction study of process variables was done by response surface methodology. It has been observed that the ChCl-EG based eutectic solvent has performed excellently in term of extraction yield, phenolics/flavonoids extraction and biological activities and can be successful used as an alternative of the conventional solvents.

P.46. SOLVENT EXTRACTION OF RHENIUM FROM SECONDARY WASTES

Humma Akram Cheema and Sadia Ilyas*

Mineral and Material Chemistry Laboratory, Department of Chemistry,

University of Agriculture Faisalabad (UAF) 38040, Pakistan

Corresponding author Email: sadiailyas1@yahoo.com

The selective recovery of rhenium from roasting dust and its subsequent stripping were investigated using the solvent extraction technique. In this study, tri-butyl phosphate as organic extractant (EXT) and metals bearing dust solution were used as the organic and aqueous feed, respectively. The extraction curve as a function of equilibrium pH depicted the maximum separation of rhenium at high acid concentration i.e., $\text{pH}_{\text{eq}(\text{ext.})} = -0.3$. The variation of extractant concentration and $\text{pH}_{\text{eq}(\text{ext.})}$ revealed the formation of $[\text{HReO}_4 \cdot 3\text{EXT}]$ adduct in the organic phase. A quantitative rhenium extraction of approximately $> 99.6\%$ was observed while contacting the metal-bearing aqueous with 0.65 mol/L EXT. A study on thermodynamic parameters indicated exothermic extraction process. The stripping of rhenium from loaded extractant as a function of pH yielded an efficient recovery of 99% at $\text{pH}_{\text{eq}(\text{strip})} \geq 3.0$ that demonstrated the potential of present work.

**P.47. ANTIHYPERGLYCEMIC, ANTIOXIDANT, HYPOLIPIDEMIC,
RENOPROTECTIVE AND HEPATOPROTECTIVE ACTIVITIES OF ZINGIBER
OFFICINALE RHIZOME IN DIABETIC RATS**

Abraisham Fatima, Fatma Hussain, Muhammad Shahid

Department of Biochemistry, University of Agriculture Faisalabad (UAF) 38040, Pakistan

Corresponding author Email: fatmauaf@yahoo.com

Z. officinale have been used worldwide as a natural medicinal product. The present study was designed to assess the antihyperglycemic, antioxidant, hypolipidemic, renoprotective and hepatoprotective efficacies of Z. officinale rhizomes in normal and alloxan-induced diabetic rats. During 21 days oral treatment trial with methanolic extract, fasting blood glucose, HbA1c, lipid peroxidation, catalase, superoxide dismutase, reduced glutathione, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, aspartate transaminase, alanine transaminase, alkaline phosphatase, glutamyl transpeptidase, total plasma proteins, urea, creatinine, albumin, total urinary protein levels were determined in normal and diabetic groups. Significant decrease in blood glucose, HbA1c, lipid peroxidation, triglycerides, total cholesterol, aspartate transaminase, alanine transaminase, alkaline phosphatase, glutamyl transpeptidase, urea and creatinine level was observed in diabetic rats treated with Zingiber officinale rhizome extracts as compared to untreated sample. Elevated catalase, superoxide dismutase, reduced glutathione concentrations were measured in diabetic samples after treatment. Variations in other biochemical parameters studied were not significant. These findings suggest that Zingiber officinale rhizomes exhibit hypoglycemic, antioxidant, hypolipidemic and hepatoprotective activities in alloxan-induced diabetic rats.

Keywords: Zingiber officinale, rhizomes, extracts, diabetic, antioxidant

P.48. VARIATIONS IN THE BUBALINE GROWTH HORMONE GENE IN THE CODING AND NON-CODING REGION

Amtul Jamil Sami, Annum Zehra and Sehrish Bilal

Institute of Biochemistry and Biotechnology University of the Punjab Lahore Pakistan.

The primary focus of animal genetics is the identification of genes which have an important role in the expression of quantitative traits. Growth hormone is the major gene which plays regulatory role in growth and metabolism of vertebrates. Several reports have identified single nucleotide polymorphism in bovine GH gene. Various such mutations have found to be associated with animal productivity. The bovine GH has been studied quite thoroughly but very little information regarding Buffalo growth hormone is available in literature. Studies report that *Bubalus bubalis* growth hormone gene has structure quite similar with bovine growth hormone. Buffalo is an important source of milk in Asia and there is a need to identify variations in the genes of buffalo GH and its possible effects in milk production. The present research was carried out to explore DNA polymorphism in buffalo growth hormone gene. In this study we amplified a 5' flanking region covering exon 1 from a local specie of *Bubalus bubalis*. As the 5' region of the GH is very important in controlling the expression of the gene and minor changes in this sequence can affect its expression in blood. The PCR amplicon was sequenced and analyzed for homology with the other available sequences in the gene bank with the help of BLAST search. Surprisingly, along with various point mutations in this region, we found that a considerable base sequence upstream exon 1 was similar to *Bos mutus* (yakQH1 chromosome 19) and did not align with reported *Bubalus bubalis* GH sequence. Only 49% of the sequenced product aligns with *Bubalus Bubalis* though 90% of the sequence aligns with *Bos mutus* yakQH1 (Accession no.CP027087.1) and *Bos indicus* GH2 gene (Accession no.AY662651.1). This report not only raises questions about the purity of the gene but also indicates mutations which may affect animal productivity like milk yield, growth regulation and carcass composition. There is a need to report these mutations so that their effects can be studied further. The complete animal history, means of semen supply or the methods used for its introduction can give clues about these findings.

Keywords: *Bos mutus*, *Bubalus bubalis*, *Bos indicus*, *Capra hircus*, growth hormone, mutation

**P.49. ELIMINATION OF DYES FROM AQUEOUS SOLUTION USING BIOCHAR
BASED ZNO NANOCOMPOSITES**

Iqra Saleem, Raziya Nadeem, Ammara Amjad, Sumaira Mumtaz, Sadaf Nazir
and Hafsa Moazzam

Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: iq.sl922@gmail.com

Dyes being released into fresh water bodies cause aquatic pollution, posing serious threat to organism's life. Minor quantities of toxic dyes can cause serious health issues resulting allergic and mutagenic diseases. Removal of such pollutants from waste water by suitable techniques is of great interest. Adsorption is one of the cost effective technique used for waste water treatment. In this research work ZnO nanoparticles were prepared using *Ficus religiosa* extract and then nanocomposites were prepared using wheat straw biochar. Present study deals with the adsorption of tartrazine dye, an anionic dye onto biomass, biochar and biochar based ZnO nanocomposites separately. Sorption experiments were performed to evaluate the effects of parameters including pH, initial concentration, contact time and dosage rate. Dye adsorption was critically dependent upon pH value with the maximum adsorption amounts occurring under acidic conditions and decreased with the increase of solution pH. The optimal pH was found to be 2.0 and optimum adsorbent dose was 0.1 g. Adsorption equilibrium data fitted well to Freundlich isotherm model in comparison to the model of Langmuir. Adsorption process was best explained by pseudo 2nd order model. Results suggested that ZnO nanocomposites has maximum uptake capacity (81.52mg/g) as compared to biomass (54.8mg/g) and biochar (67.5mg/g). Surface morphological characteristics were analyzed by SEM/EDX.

Keywords: Pollutant, dye, adsorption, biochar, nanoparticles, nanocomposites.

P.50. IDENTIFICATION OF BZIP GENES IN COTTON

Atika Tariq, Tayyaba Shaheen*, Hira Tahir and Farrukh Azeem

Corresponding author Email: tayaba_pgmb@yahoo.com

Basic leucine zipper (bZIP) is one of the largest transcription factor gene families in plants, involved in regulating environmental stress responses, growth and developmental processes of plants. bZIP TFs are of great importance due to their potential role in plant's natural host defense mechanism to survive lethal stress exposures. However, the knowledge related to structural and the functional characterization of the bZIP gene family in plants is insufficient. Identification and characterization of bZIP genes in cotton is particularly deficient. *G. ramondii* and *G. arboreum* are considered to be the ancestor of *G. hirsutum*. *G. arboreum* (Desi cotton) has contributed as one of the parental genome of present day cotton (*G. hirsutum*) and its genome has many favorable traits like resistance to biotic and abiotic stresses. Intron-less genes are unique genes in context of having no introns in them. This study was aimed to identify intron-less bZIP genes in *G. arboreum*, *G. hirsutum* and *G. raimondii* to highlight any variations. Twenty-one intron-less gene sequences in *G. ramondii*, *G. arboreum* and *G. hirsutum* were used for in silico analysis. All the selected bZIP intron-less genes were found to possess bZIP domain using NCBI's Conserved Domain Search (CDD). Total 19 Intron-less bZIP proteins also shown to possess additional domains other than bZIP domain that depicts their versatility. Using EBI's Clustal Omega tool for multiple sequence alignment, triads of intron-less bZIP genes from *G. arboreum*, *G. hirsutum* and *G. ramondii* were aligned. Variations among the sequences of three species were analyzed to decipher any evolutionary changes and the proposed ancestor-descendant relationship of three *Gossypium* species. Seven genes were found highly conserved across the three species and only two genes showed high divergence. This study laid a foundation for further research on the bZIP gene family in cotton, their structural analysis and further utilization.

Keywords: Cotton, bZIPs, intron-less, abiotic stress

P.51. EFFICACY OF ANTIBIOTICS, CURCUMIN LONGA NANOPARTICLES, AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AGAINST WOUND BASED MULTIPLE DRUG-RESISTANT STAPHYLOCOCCUS AUREUS AND E. COLI

Iqra Sarwar¹, Ashar Mahfooz¹, Ambreen Ashar², Ijaz Saleem¹, Amjad Islam Aqib³, Muhammad Shoaib⁴, Tayyab-ur-Rehman⁴, Muhammad Fakhar-e-Alam Kulyar¹, Masood Sadiq¹, Ahsan Raza Gilani¹, Muhammad Ahsan Anawar¹, Zeeshan Ahmed Bhutta¹

¹Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad

²Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur-Pakistan

³Government College University for Women, Faisalabad

⁴Institute of Microbiology, University of Agriculture, Faisalabad

Corresponding author Email: amjadwaseer@gmail.com

Involvement of secondary bacterial infection from both gram positive (*S. aureus*) and gram-negative (*E. coli*) classes of bacteria significantly delay wound healing. The scenario requires effective and alternative therapeutics to deal with wound healing challenges. Current study was planned to check prevalence of multiple drug resistant *Staphylococcus aureus* and multiple drug resistant *E. coli* from wound of animals, their drug response to antibiotics, to evaluate effect of nanoturmeric (NT) and raw turmeric (RT), and to examine efficacy of non-steroidal anti-inflammatory drug (NSAID) in combination with antibiotic. Wound samples (n=150) were aseptically collected from the animals presented at veterinary teaching hospital, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad. Microbiological and biochemical assays were performed to isolate *Staphylococcus aureus* and *E. coli* while multiple drug resistance was assessed by checking response to antibiotics using Kirby Bauer's disc diffusion method. Nanoturmeric and raw turmeric was applied against MDR isolates. MDR resistant to amoxicillin were also tested against aspirin alone and in combination with amoxicillin. The data was analysed by non-probability testing at 5% probability using SPSS statistical computer program. Study found 22.85 and 21.42% *E. coli* and *Staphylococcus aureus* from wound of animals. MDR *Staphylococcus aureus* were found 36.36, 57.14, and 100% sensitive to oxytetracycline, chloramphenicol, trimethoprim-sulphamethoxazole, respectively. On the other hands, percentage of sensitive isolates of MDR *E. coli* against ampicillin, oxytetracycline, trimethoprim-sulphamethoxazole, chloramphenicol, and ciprofloxacin were noted to be 0.00, 80, 86.66, 87.50, and 66.66%, respectively. These MDR isolates when tested

in-vitro against nanoturmeric @ 10mg/mL, 1mg/mL, and 0.1mg/mL presented 16mm, 13mm, and 12 mm of zone of inhibition. On the other hands, raw turmeric at concentration mentioned above displayed 14mm, 11mm, 10mm zone of inhibitions around MDR *S. aureus*. Aspirin, a non-steroidal antinflammatroy drug, did not inhibit growth of *S. aureus* and *E. coli* when used alone but in combination with amoxicillin, increased zones of inhibition. The study concluded higher prevalence of multiple drug resistant bacterial contaminants, appreciable antibacterial response of nanoturmeric, and synergistic combination of NSAID with antibiotic against MDR isolates.

Keywords: *S. aureus*, *E. coli*, nanoturmeric, aspirin, antibiogram,

**P.52. BIODEGRADATION OF VAT ACTA FFB BY *PHAEOLUS SPADICEUS* AND
STUDY OF MANGANESE PEROXIDASE ENZYME**

Kianat Javaid¹, Raja Tahir Mahmood¹, M. Javaid Asad², Imran Ali¹ and Rimsha Ashraf¹

¹Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 (AJK) Pakistan

²Department of Biochemistry, PMAS-Arid Agriculture University Rawalpindi, Pakistan

Corresponding author Email: rajatahir87@gmail.com

Dye is a colored substance used to impart permanent color to other substances. Its most important use is in coloring textile fibers and fabrics. Textile industry discharge a huge amount of dye containing waste water which pollutes water, soil and also have adverse effect on human health, animal and plants. Imperfection of dyeing process, approximately 15-20 % of the synthetic dyes is released into the industrial effluent, causing serious environmental problems. Removal of dyes from dye containing waste water must be done by either physico-chemical or biological methods. The current study was designed to explore the potential of *Phaeolus spadiceus* to degrade VAT ACTA FFB textile dye and study of enzyme manganese peroxidase. The result showed that maximum biodegradation was 81 % at temperature 35°C, pH 5.6 after 5.4 days. Manganese peroxidase was characterized for optimum temperature, pH and kinetics parameter. Optimum temperature and pH of manganese peroxidase were 30°C and 4.5 respectively. The value of Vmax (1666.66 uM/ml/min) and Km (5.16 mM). Higher enzymatic activity related with more biodegradation indicated that this enzyme involved in this process. This study concluded that *Phaeolus spadiceus* is the potential microorganism for textile dye biodegradation and this will help to eliminate environmental pollution.

Key Words: *Phaeolus spadiceus*, Biodegradation, textile effluent, manganese peroxidase

P.53. LABELING AND BIODISTRIBUTION OF ^{99m}Tc -PREDNISOLONE

Muhammad Bilal Butt¹, Tanveer Hussain Bokhari¹, Muhammad Daud², Samina Roohi²
and Saira Hina³,

1 Department of Chemistry, Government College University, Faisalabad, Faisalabad 38000,
Pakistan

2 Isotope Production Division, Pakistan Institute of nuclear Science and Technology P.O
Nilore, Islamabad, Pakistan

3 Government College Women University, Faisalabad, Pakistan

Prednisolone is used in the treatment of inflammation, allergies, rheumatoid and certain cancers as well. Prednisolone was used for radiolabeling with Technetium-99m. Radiochemical yield >90 % was obtained by adopting optimized protocol. For the preparation of ^{99m}Tc -labeled prednisolone optimized parameters such as stannous chloride, amount of ligand, pH, and incubation time were evaluated. High radiochemical yield was confirmed by paper chromatography. For biodistribution purpose, male white Sprague-Dawley New Zealand mice were used. The activities of ^{99m}Tc -prednisolone in different organs like stomach, heart, liver, spleen, intestine, lungs, kidney and femur were also evaluated.

P.54. ENTOMOPATHOGENIC FUNGI: A NOVEL CONTROL AGENT TO COMBAT ACARICIDAL RESISTANCE

Muhammad Zeeshan^{1*}, Muhammad Sohail Sajid^{1,2}, Muhammad Abdullah Malik¹, Kashif
Hussain¹, HaroonAhmad¹

¹Department of Parasitology, University of Agriculture, Faisalabad.

²One Health Laboratory, Center for Advanced Studies in Agriculture and Food Security
(CAS-AFS), University of Agriculture, Faisalabad

Corresponding author Email: dr.zee474@gmail.com

Ticks are hematophagous parasites which are present worldwide, creating serious life threats to both domestic and wild animals. They effect either directly causing poor health and decrease in production or indirectly to human beings through milk and meat consumption. Moreover, these vectors are responsible for the transmission of many bacterial, viral and protozoal diseases which have zoonotic potential e.g. Arbovirus causing encephalitis, Crimean Congo hemorrhagic fever, theleriosis, babesiosis, lyme disease etc. There are various acaricides including: arsenics and chlorinated hydrocarbons have been reported effective against ticks. But, due to continuous exposure, misuse, lower dose administration, use of outdated acaricides and lack of awareness of local farmers these vectors, evolved resistance against these acaricides. To overcome increasing resistance, alternate control methods have been reported to be used against ticks. Biological control is one of these alternate control methods. The arthropod vectors can be controlled using entomopathogenic fungi, entomopathogenic nematodes and nanoparticles. Now a day, entomopathogenic fungi are being used to control ticks. Entomopathogenic fungi has been used worldwide for biological control of ticks especially *Rhipicephalus microplus*. These can be isolated from soil by N-acetylguanidine monoacetate (dodine) and can be cultured at Potato dextrose agar plus yeast (PDAY). There are two genera *Beauveria* and *Metarhizium* which have been investigated for their efficacy against arthropods. In Pakistan there is not enough work has been done in this aspect. The aim of this study is to evaluate the virulence of fungal isolates in vitro as well as in vivo in tick prevalent areas of Pakistan. The results of the study will provide the information regarding fungal isolates effective against ticks. Hence, it will provide an alternate control in the presence of acaricidal resistance which ultimately reduce the ticks and tick-borne diseases in the study area.

Keywords: Ticks, Entomopathogenic fungi, Biological control, Zoonotic potential

**P.55. OPTIMIZATION OF QUERCETIN LOADED POLYMER COATED
IRON OXIDE NANOPARTICLES, THEIR THERAPEUTIC POTENTIAL
AND SUB-ACUTE TOXICITY STUDIES**

Marryum Ashraf¹, Bushra Akhtar¹, Faqir Muhammad^{1*}, Yasir Javeed², Ali Sharif³,

Amreen Ashraf¹

¹Institute of Pharmacy, Physiology and Pharmacology, ²Department of Physics,

University of Agriculture, Faisalabad, Pakistan

³-Department of Pharmacy, The University of Lahore, Pakistan

Iron oxide nanoparticles (IONPs) have gained attraction due to several properties and can be aimed to provide site/tissue specific drug delivery. But they are toxic for body. IONP's prepared through co-precipitation method have profound properties which are enhanced by incorporating quercetin. We aimed to synthesize quercetin loaded IONPs because of its poor permeability and solubility. To improve quercetin bioavailability, these IONPs were coated then with polyethylene glycol (PEG) and chitosan. IONPs were characterized for size, potential and XRD and FT-IR and entrapment efficiency. Quercetin loaded IONPs performed an important part in shielding cells from oxidative damage carried out by free radicals and inhibited lipid per oxidation as these IONPs showed 68.12% DPPH scavenging activity. Along with therapeutic applications, there was a focus on *in vivo* toxicological effects of bare IONPS VS polymeric coated IONPs to explore safety profiles of this nano-formulation by approximating alterations of blood cell parameters, liver enzymes functioning or variation in histopathological features of organs of experimental animals after delivery of the quercetin loaded IONPs. Considerable difference between efficacy and toxicity values, showed that bare IONPs treated rats exhibited more toxicity while chitosan coated were the safest one in various aspects.

Keywords: Quercetin, Iron oxide, Nanoparticles, Chitosan, Toxicity

P.56. RECOVERY OF MINERAL ACID FROM INDUSTRIAL WASTES

Muhammad Ahmad Muhsan and Sadia Ilyas

Mineral and Material Chemistry Laboratory, Department of Chemistry, University of
Agriculture Faisalabad (UAF) 38040, Pakistan

Corresponding author Email: sadiailyas1@yahoo.com

Disposal of acidic effluents containing high chlorides and sulfates concentration is a serious problem for ecology, environment and human health. Therefore, the recovery of mineral acids with tri-butyl phosphate (EXT) has been investigated from the effluent streams in presence of metal (Na^+ , Ca^{2+} and Mg^{2+}) chlorides, sulfates and compared to the behavior exhibited by HCl and H_2SO_4 extraction in absence of metal chlorides and sulfates. The comparative study on parametric influence showed a higher acid removal in presence of additional metal chlorides and sulfates in aqueous solution. The variation of EXT, chloride and sulphate ions concentration clearly demonstrated their influence on acid extraction and revealed the formation of $\text{EXT}\cdot\text{HCl}$, $\text{EXT}\cdot\text{H}_2\text{SO}_4$ adduct nearly at 1:1 ratio, yielding equilibrium constant ($\log K_{\text{ext.}}$) values -0.428 and -0.316 in absence and presence of additional metal chlorides and sulfates respectively. The McCabe-Thiele plots indicated the requirement of four and two extraction-stages under counter-current flow for acid solutions in absence and presence of metal chlorides and sulfates respectively at a same phase ratio of 0.8:1. The maximum loaded organics in both conditions of aqueous feed were subsequently stripped with water, recovering ($> 99\%$) hydrochloric acid and sulphuric acid can be reused. The acid removal from effluent stream is a sustainable process to control the chlorides and sulfates discharge and limiting the consumption by its possible recovery and recycling.

P.57. ANTIBACTERIAL AND ANTIOXIDANT SCREENING OF CALOTROPIS PROCERA L.

Naima Kanwal, M. Altaf Hussain, Sana Qasim and Raja Tahir Mahmood
Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 (AJK) Pakistan

Leaves of *Calotropis procera* (Family: Asclpiadaceae) were extracted with various organic solvents. These extracts were subjected to the potential antioxidant and antibacterial activities. The antioxidant potential of the extracts was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. IC₅₀ of the ethanol extract of *Calotropis procera* was 1.44 which conferred the strong antioxidant activity of the plant. For antibacterial activities of solvents extracts were assessed against two Gram-positive and two Gram-negative bacteria by using disc diffusion method. The ethyl acetate, chloroform and ethanol crude extracts of selected plant part had significant antibacterial activities on both Gram positive and Gram negative bacteria. The ethyl acetate and ethanol crude extracts of leaves *Calotropis procera* exhibited prominent activities against Gram positive and Gram negative bacteria used in comparison to other extract which had moderate activity against all the tested bacteria. The antibacterial activities of the crude extracts of the selected plant part were more active against Gram positive bacteria than Gram negative bacteria. The standard reference antibiotic Rifampicine was used as positive control.

Keywords: *Calotropis procera*, antioxidant, antibacterial

**P.58. ANTIMICROBIAL POTENTIAL OF SELECTED MEDICINAL PLANTS
AGAINST PATHOGENIC BACTERIA**

Sidra Ashraf, Hina Fatima and Muhammad Shahid

Department of Biochemistry, University of Agriculture Faisalabad

Plants considered to be very important due to their texture and many other properties such as medicinal, antimicrobial, antifungal, antioxidant and anticancer properties. The present research project was planned to evaluate the antimicrobial potential and antibiofilm potential of methanolic extract of four medicinal plants i.e. *Cuminum cyminum*, *Cichorium intybus*, *Elettaria cardamomum* and *Allium sativum*. Antimicrobial potential was determined by well diffusion method and biofilm inhibition was carried out through microplate method. All experiments were performed in triplicates. Data was expressed as mean and standard deviation ($\pm$ standard error). The antibiofilm and antibacterial activities done against four species of bacteria which includes gram-positive and gram-negative bacteria. Results of all activities are shown that *Allium sativum* has maximum antibiofilm potential i.e. 43.67 ± 1.23 against *S. aureus* bacteria and all other medicinal plants also have significant antibiofilm potential. The cumin has maximum antibacterial potential i.e. 24mm (inhibition zone) against *S. aureus* bacteria.

P.59. SYNTHESIZATION OF BIO-ACTIVE SILVER NANOPARTICLES BY USING CUMIN EXTRACT

Rabeea Muzaffar¹, Afsar Bano^{2*}, Tahira Iqbal¹, Rafia Rehman³ and M. Shahid¹

¹Department of Biochemistry, ³Department of Chemistry,
University of Agriculture, Faisalabad, Pakistan

²Department of Physics, COMSATS Institute of Information Technology, Islamabad

Corresponding author Email: Bano_afsar@yahoo.com

Bioactive nanoparticles are those particles which have biological effects and enhanced therapeutic efficacy. In the present study, cumin was purchased from local market of Faisalabad. The extract of the spices was prepared using water as a solvent. The extract so obtained was used for silver nanoparticles preparation by green method. The silver nitrate (AgNO₃) aqueous solution as a precursor was employed in the silver nanoparticles synthesis process. Silver nanoparticles were prepared in dark and by using the light. The comparison was developed for characterization and activities. In each procedure, change in the colour of the reaction mixture from light yellow to dark brown was observed periodically. UV-Vis spectrophotometer was used to analyze the resulting colloidal solution of silver nanoparticles which shows that more homogenize particles were formed in the presence of light. The estimation of antimicrobial activity of bioactive silver nanoparticles was carried out using disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. Maximum activity against *Escherichia coli* was shown by that particles which produced in the presence of light then by the nanoparticles prepared in dark and reverse trend was observed in case of *Staphylococcus aureus*.

Keywords: Silver nanoparticles, Cumin, Disc diffusion method, AgNO₃, UV-Vis spectroscopy

P.60. ELECTROCHEMICAL SENSING OF AMINO ACID AT THE 2, 4-DINITROPHENYL HYDRAZINE MODIFIED CARBON PASTE ELECTRODE: A CYCLIC VOLTAMMETRIC STUDY

Rahat Nawaz, Taniya Iqbal and Saadat Majeed*

Division of Analytical Chemistry, Institute of Chemical Sciences,

Bahauddin Zakariya University Multan

Corresponding author Email: saadat.majeed@bzu.edu.pk

2, 4 Dinitrophenyl Hydrazine modified carbon paste electrode (2, 4 DNPHMCPE), was developed for the electrochemical investigation of amino acid by using cyclic voltammetric technique. The modified carbon paste electrode shows remarkable electro activity toward amino acid as compare to bare carbon paste electrode (BCPE) at optimized condition. The best oxidative peak current is obtained at the concentration of 0.6×10^{-4} in phosphate buffer solution (PBS) of physiological pH 7.4. The analytical signal of glycine, glutamic acid, and aspartic acid were obtained at +0.8V, +0.6V, +0.5V at a scan rate 0.005V/s. In the multiple scan there is absence of cathodic peak with which determined the reaction is irreversible. 2,4 DNPH modified carbon paste electrode gave a linear response between concentration of amino acid and its anodic peak (E_{pa}) current in the range within 0.1×10^{-4} - 0.6×10^{-4} M and the limit of detection (3S/M) 0.4×10^{-5} M correspondingly. Study of scan rate effect shows that electrode process was diffusion controlled. The participation of protons and electrons in the oxidation of amino acid was found to be equivalent which determines that electrode is good precision, accuracy and prices. This method can be used in development of a sensor for sensitive sensing of amino acid.

P.61. BIOLOGICAL AND CHEMICAL PROFILE OF ENDOPHYTIC MICROBES UNDER SALT STRESS

Hina Fatima¹, M. Shahid¹, M. Nauman Gulzar¹, Muhammad Zubair Ghouri^{2,3}
and Aftab Ahmad^{1,2}.

¹Department of Biochemistry, ²Center for Advanced Studies in Agriculture and Food
Security (CAS-AFS), ³Centre of Agricultural Biochemistry and Biotechnology (CABB),
University of Agriculture, Faisalabad (UAF), Pakistan.

Corresponding author Email: hinafatimauaf@yahoo.com

Endophytic microbes produce a large number of secondary metabolites which are useful to host plant and also have pronounced biological activities. The present research work had been designed to evaluate the chemical and biological potential of endophytic microbial extracts cultured under normal and salt stress. All strains were found to be salt (4%) tolerant. Secondary metabolites of endophytes were extracted with ethyl acetate and tested for their biological and chemical potential. Overall results of biological potential showed that endophytic extracts are rich in polyphenolic contents and have good antioxidant capacities under normal and salt stress conditions. Extracts with higher antioxidant potential also prevent DNA from damaging effects of hydrogen peroxide. Antimicrobial potential of secondary metabolites was evaluated through disk diffusion method. Extracts were effective against both Gram negative (*Pasterulla multocida*, *Escherichia coli*) and Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacteria, while some extracts also inhibit growth of pathogenic fungi *Fusarium oxysporum* and non-pathogenic fungi *Ganoderma lucidum*. Extracts also prevent biofilm formation and act as strong inhibitor of *Staphylococcus aureus* biofilm. Secondary metabolites of extracts also tested for thrombolytic potential and showed significant clot lysis activity. Extracts were found to be least toxic towards human RBCs. In chemical screening of secondary metabolites extracts were analyzed by UV-Vis spectroscopy and high-performance liquid chromatography. Result of UVVIS spectroscopic analysis suggests the presence of phenolics and flavonoids in the ethyl acetate extract of bacteria. UV-VIS profiling of extracts showed different peaks ranging from 210nm to 360nm which are characteristics of phenolic acids and flavonoids. HPLC analysis was used for identification and quantification of phenolic and organic acids. Results showed presence of different organic and phenolic acids in all extracts, and their types and contents were found to be different under different culture conditions. Most abundant phenolic acids were gallic acid, chlorogenic acid, vanillic acid and cinnamic acid, while pyruvic acid, citric acid and malic acid were main organic acids identified in ethyl acetate extracts. The results obtained were analyzed through one-way analysis of variance (ANOVA).

Keywords: Endophytic bacteria, antimicrobial potential, antioxidant potential, cytotoxicity

P.62. CHARACTERIZATION OF MANGO (*MANGIFERA INDICA* L.) FRUIT'S TRANSCRIPTOME AND METABOLOME DURING DEVELOPMENT

Zainab Khanum¹, Martín E. Tiznado-Hernández², Syed Ghulam Musharraf¹, Arslan Ali¹
and Ishtiaq A. Khan*¹

¹International Center for Chemical and Biological Sciences, University of Karachi,
Karachi- 75270, Pakistan.

²Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en
Alimentación y Desarrollo, A. C. Hermosillo, Sonora, México.

Corresponding author Email: ishtiaqchemist@gmail.com

Chaunsa White mango fruit is one of the best export quality mango variety of Pakistan. This variety is well known for its taste and aroma globally. However, there is very limited information available on this cultivar's unique biochemical composition. Here we report temporal dynamics of mango fruit's transcriptome by real time quantitative PCR analysis by focusing on genes involved in the sweetness, aroma and color biosynthesis, along with the genes which regulate abiotic stress, fruit standing and circadian rhythm control. Primers for RT-qPCR analysis were designed using transcripts from **Database of mango exocarp genes including 107,744 annotated unigene** (Tafolla-Arellano, et al. 2017). The ripe Chaunsa mango fruit pulp's metabolome was also characterized by GC/MS analysis in this study. The present research work has revealed eleven differentially expressed genes during mango fruit's development. The differentially expressed genes characterized include: glycolytic and starch biosynthesis gene (Cai, Li et al. 2016), color producing "Chalcone synthase" gene (Nakabayashi, Yonekura-Sakakibara et al. 2014), senescence associated gene (Wehner, Balko et al. 2016), stress regulating "Nucleoredoxin" protein gene (Kneeshaw, Keyani et al. 2017), reactive oxygen species control genes (Selmi, Rtibi et al. 2017), aromatic amino acid biosynthesis gene (Langer, Jones et al. 2014), aroma producing "Linalool synthase" gene (Iwasaki, Zheng et al. 2016) and circadian rhythm control nuclear factor protein gene "GIGANTEA" (Dalchau, Baek et al. 2011). Metabolome characterization has revealed the presence of 37 unique aroma and flavor producing compounds such as Terpenes, Terpenoids, reducing-sugars and Phenols. Geraniol is a unique acyclic monoterpene alcohol found in essential oils. **Geraniol** is present in Chaunsa mango fruit pulp. This compound gives sweet, floral and citrus aroma to pulp (Li, Lim et al. 2013) and has several health benefits such as anti-enteric pathogen activity (Perez-Lewis, Yegin et al. 2018) and anti-cancer activity against several Cancers (ChO, So et al. 2016).

P.63. DNA BARCODING: A USEFUL TAXONOMIC TOOL FOR SPECIE IDENTIFICATION

Rana Hamid Ali Nisar,^{1*}, Muhammad Sohail Sajid^{1, 2}, Mahvish Maqbool¹, Muhammad Abdullah Malik¹

¹Department of Parasitology, University of Agriculture, Faisalabad, Pakistan

²Center for Advanced Studies in Agriculture and Food Security (CAS-AFS) University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: drhamidali2010@gmail.com

DNA barcoding is very common technique in which genetic markers are used to detect individual and specific species. DNA barcoding provide a well-organized method for species identification and contribute strongly to taxonomic research. First time DNA barcoding studied in birds for identification of species. As the number of DNA barcode sequences collected this data gives a distinctive genomics perspective with wide implications. DNA barcoding is helpful because it provides background information that will be helpful in the taxa selection for further investigation. DNA sequences become the important source of new information for enhancing our understanding of evolutionary and genetic relationships because of modifications in sequencing and computer based technologies. The footprints of comparative sequence investigation are now seen in almost all branches of the biological sciences, from development to epidemiology. Two branches of biology developed the tools and applications employed to evaluate biological relationships with sequences of DNA which includes population genetics and molecular phylogenetics. These branches focus on various levels of organization. Molecular phylogenetics specifically deal with evolutionary relationships between deeper clades while population genetics target difference within and between populations of a single species. DNA barcoding holds a middle ground as it provides complete coverage for species, but focuses on their description instead of their relationships. DNA barcoding based on the hypothesis that a short standardized sequence can discriminate members of a species because genetic difference between species overshoot within species. Now a days DNA barcoding successfully used in many large groups of animals, like cowries, spiders, birds, fish and several arrays of Lepidoptera. Additionally, DNA barcoding systems are also established for some other group of organisms like bacteria, macro algae, plants, fungi and protists. DNA barcoding datasets are made up of short DNA sequences from many individuals of species. Typically, five to ten individuals per species, but these numbers will increase in the future.

Keywords: DNA barcoding, phylogenetics, DNA barcode, Taxonomy

**P.64. ANTIBACTERIAL AND ANTIOXIDANT SCREENING OF
CANNABIS SATIVA L**

Sana Qasim, M, Altaf Hussain, Naima Kanwal

Department of Biotechnology, Mirpur University of Science and Technology (MUST),
Mirpur-10250 (AJK) Pakistan

The aim of the present study was to evaluate the antibacterial potential of *Cannabis sativa* L. extracts against clinically important Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and their antioxidant potential. The extracts of selected plant with three solvents viz. ethanol, ethyl acetate and chloroform were used to assess the biological potential. The commercially available antibiotic i.e. Rifampicin was used as positive control. Organic solvent extracts were screened for antimicrobial activities by disc diffusion method. The antioxidant activity was determined by using 2, 2-diphenyl-1-picrylhydrazylhydrate (DPPH) assay. The ethyl acetate and ethanol crude extracts of leaves *Cannabis sativa* exhibited prominent activities against gram positive and gram negative bacteria used in comparison to other extracts which had moderate activity against all the tested bacteria. The antibacterial activities of the crude extracts of the selected plant parts were more active against gram negative bacteria than gram positive bacteria. The study revealed that leaves of *C. sativa* possess broad spectrum antibacterial activity and natural antioxidants that can be of considerable pharmaceutical importance as the chloroform extract proved to be more potent source of antioxidant activity with IC₅₀ 4.9048625. Moreover it was confirmed that the antioxidant activity was dose dependent as the value of R² is 0.9628.

Keywords: *Cannabis sativ*, Antioxidant, antibacterial, ethylacetate

P.65. BIOPROCESSING OF LOW GRADE CHALCOPYRITE

Shafaq Masud and Sadia Ilyas*

Mineral and Material Chemistry Laboratory, Department of Chemistry, University of
Agriculture Faisalabad (UAF) 38040, Pakistan

Corresponding author Email: sadiailyas1@yahoo.com

With the world wide increasing demand of copper in building material, transportation, electronic products industries the high grade sources of copper are going to depleted with an alarming rate and there is an absolute necessity of time to process copper from low grade and secondary sources. On the other side, the extraction of copper by conventional pyrometallurgical processing need high cost of fuel and large operation sites. The low grade sources like low grade copper containing sulfidic minerals are less economical with these practices. So current study was conducted to process low grade sulfide ore of copper with bio hydrometallurgical routes. Experiments were conducted with *Penicillium chrysogenum* strain KBS₃. Approximately 48% Cu was leached out in one step bioleaching. In the spent medium leaching 97% Cu was extracted in 14 days. Cu extraction yield was much lower in one step bioleaching 48% than spent medium leaching 97% and chemical leaching 98% with organic acids such as citric, oxalic, tartaric, and gluconic acids. Leaching by adapted consortium of *Sulfobacillus thermosulfidooxidans* and *Thermoplasm acidophilum* in shake flasks, batch bioreactors and in continuous bioreactors at optimum temperature of 45 °C, residence time 24 hours, initial pH 2.5, agitation speed 180 rpm and particle size of 120 µm. Approximately 85% Ni, 88% Cu, 68 % Fe and 83 % Zn were leached out in continuous reactors with microbial consortium while 93% Ni, 89% Zn, 85% Cu and 75% Fe were leached out in batch bioreactors.

**P.66. SYNTHESIS OF STARCH BASED BIOPLASTIC FROM MANGO SEEDS
REINFORCED WITH CARBOXY METHYL CELLULOSE (CMC) USING
SORBITOL AS A PLASTICIZER**

Sarmad Ahmad Qamar^{1, *}, Muhammad Asgher¹, Nimrah Khalid¹, Muhammad Jahangeer²

¹ Industrial Biotechnology Lab., Department of Biochemistry, University of Agriculture,
Faisalabad, Faisalabad-38000, Pakistan

² Department of Biochemistry, Government College University Faisalabad, Faisalabad-
38000, Pakistan

Corresponding author Email: sarmad_qamar@live.com

The accumulation of petrochemical-based plastics, primarily from packaging stuffs, is causing a serious threat to our environment. Bioplastics are just like conventional plastics but will be biodegrade by the activity of microorganism into carbon dioxide and water. Starch is a natural bio macromolecule that can be used for bioplastic production. The addition of reinforcements has been shown to improve mechanical properties of plastics. This study was designed to investigate mango seed starch, carboxy methyl cellulose (CMC) as a filler and sorbitol as a plasticizer on elongation at break, tensile strength and surface morphology (SEM), of the resulting biopolymeric films. In this study films were obtained using mango seed starch 10 grams, with the varying concentration of CMC 0; 2; 4; 6; 8; and 10 wt%, and plasticizer 0; 5; 10; 15; 20; and 25% by wt. Heating temperature for resulting bioplastic solution was kept 90°C for 30 minutes. After incubation period resulting bioplastic films were investigated for elongation at break, tensile strength and surface morphology (SEM). Best semi-transparent bioplastic films were obtained on the use of 6% CMC and 15% sorbitol, with a tensile strength of 6.768MPa, percent elongation at break 47.423%.

**P.67. BIOLOGICAL PRETREATMENT OF RICE STRAW BY LIGNINOLYTIC
BACILLUS SP. STRAINS FOR ENHANCING BIOGAS PRODUCTION**

Tawaf Ali Shah^{ab}, Charles C. Lee^c, William J. Orts^c and Romana Tabassum^{ab*}

^aNational Institute for Biotechnology and Genetic Engineering
(NIBGE), Faisalabad, Pakistan

^bPakistan Institute of Engineering and Applied Science (PIEAS), Islamabad, Pakistan

^cUSDA-ARS-WRRC, Bioproducts Research Unit, 800 Buchanan Street, Albany, CA, 94710,
USA

Corresponding author Email : tawafbiotech@yahoo.com.

Fermenting agricultural biomass, such as rice straw, to methane is a promising solution to an ongoing waste problem. However, the biomass must first be pretreated to break down lignin thereby increasing accessibility of the substrate to fermentative organisms. Biological pretreatment by microorganisms represents a potentially economical strategy to prepare the biomass for fermentation. We isolated seven candidate ligninolytic *Bacillus* sp. strains based on their robustness for lignin degradation. The production of the ligninolytic enzymes from these strains was characterized and optimized. The bacterial strains were tested for their ability to pretreat rice straw and increase the yield of biomethane fermentation. It was determined that using mixed combinations of bacterial cultures was more effective than using individual strains. Overall, the study demonstrates the potential of using these *Bacillus* sp. strains as robust biocatalysts for processing lignocellulosic waste biomass.

Significance. Newly isolated *Bacillus* sp. strains demonstrated an efficient degradation of lignin, Azure B dye, fast hydrolysis of rice straw, and improved biogas yields. This research suggests that *Bacillus* sp. strains could be a source of novel enzymes and an ideal candidate in bioprocessing of complex substrates”.

Keywords: *Bacillus* sp. strain; lignin degradation; anaerobic digestion; delignification; lignocellulosic biomass.

**P.68. PHYTOCHEMICAL AND THERAPEUTIC INVESTIGATIONS OF CITRUS
PARADISI (GRAPE FRUIT) PEEL ESSENTIAL OIL**

Yahya Amjad¹, Faqir Muhammad*¹, Bushra Akhtar¹, Muhammad Irfan Anwar², Bilal
Aslam¹, Junaid Ali Khan¹, Muhammad Naeem Faisal¹

1-Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture,
Faisalabad, Pakistan

2-Faculty of Veterinary Science, Bahauddin Zakariya University, Multan, Pakistan

Corresponding author Email: Yahyaamjad40@gmail.com

Many fruits and vegetable peels have therapeutic potentials. Medicinal plants are used largely in treatment and prevention of different diseases as these drugs are cheaper and have very less side effects than the synthetic drugs. The objective of the this study was to investigate the grapefruit peels essential oil for its qualitative phytochemical analysis, antioxidant activity, and its antibacterial activity against different strains, in-vivo wound healing activity, acute skin irritation studies and GC/MS analysis of the essential oil for its chemical composition. Qualitative phytochemical investigation revealed the presence of alkaloids, flavonoids and saponins. Antioxidant activity was evaluated through total phenolic contents, along with total flavonoid contents, DPPH scavenging assay and reducing power assay. Essential oil showed highest total phenolic contents and lowest reducing power assay. Antibacterial activity was evaluated through disc diffusion method and its showed minimal activity against tested strains. In-vivo wound healing activity was recorded on rabbits after giving surgical incision wound. Grape fruit essential oil showed good wound healing activity in comparison to control treatment (pyodine). Acute skin irritation (Draize's scoring) was observed 24h prior to the application of essential oil which shows non-significant results. GC/MS analysis revealed the presence of different compounds like limonene and triacetin compounds. The study showed the presence of various bioactive compounds having potential therapeutic potential.

Keywords: medicinal plants, Grapefruit, Antibacterial activity, wound healing

P.69. DNA BARCODING: A NEW INSIGHT INTO BIODIVERSITY OF ORGANISMS

Usman Talib^{1*}, Muhammad Sohail Sajid^{1,2}, Muhammad Abdullah Malik¹, Muhammad Uzair Asgahr¹ Tean Zaheer¹, Sami Ullah Khan Bahadur³

¹Department of Parasitology, ²One Health Laboratory, Center for Advanced Studies in Agriculture and Food Security (CAS-AFS), ³Department of Pathology, University of Agriculture, Faisalabad.

Corresponding author Email: usmangill007@gmail.com

Identification of species is a fundamental part of recognizing and describing biodiversity as we monitor the biological effect of climate change to prevent accelerated destruction of habitat. Morphological identification is limited due to phenotypic plasticity, genetic variation and complexity in morphology. DNA barcoding has been ascertained a standard molecular identification method for speciation of an organism, by using PCR of short and standardized DNA segment of 400-800 bp. Mitochondrial cytochrome oxidase I (COI) is preferred over nuclear genome as it is maternally inherited, with high mutation rate and no introns to avoid recombination problem which result in high degrees of intra-specific polymorphism. Many studies have declared that DNA barcoding method provides 95-97% accuracy at species level, thus help in preventing mislabeling of old species with new one. DNA barcoding will also enhance the capacity in many areas for conversation of species and provide new insights in nature of species. However, ecological and behavioral characteristics are still important and used along with the information. Keeping in view the wide range of applications of DNA barcoding it can be hypothesized that it could be a useful tool for the identification of various arthropods fauna, prevalent in Pakistan. The results of this identification will not only add up the data in existing genomic information of the arthropods but, also paves the way to identify emerging species of arthropods.

Keywords: Identification, DNA barcoding, arthropods, biodiversity.

P.70. EXTRACTION OF BIOACTIVE COMPOUNDS FROM MENTHA ARVENSIS L. USING CHOLINE CHLORIDE AND ETHYLENE GLYCOL (EG) BASED EUTECTIC SOLVENT: OPTIMIZATION AND BIOLOGICAL ACTIVITIES

Zubera Naseem¹, Muhammad Zahid^{*1}, Muhammad Asif Hanif¹, Muhammad Shahid²

¹ Department of Chemistry, ² Department of Biochemistry,

University of Agriculture, Faisalabad

Corresponding author Email: rmzahid@uaf.edu.pk

Choline chloride (ChCl) and ethylene glycol (EG) based deep eutectic solvent (DES) was used for the extraction of bioactive compounds from *M. arvensis* L. using maceration (ME), ultrasound assisted (UAE) and microwave assisted (MAE) extraction techniques. The optimization of extraction parameters and their interactive influence was evaluated through response surface methodology (RSM). The highest total phenolic contents (125 mg GAE/g), total flavonoids contents (95 mg QE/g) and 2, 2-diphenyl-1- picrylhydrazyl (DPPH) radical inhibition (97%) was assessed for UAE. The antibacterial activity of extracts was evaluated against *Staphylococcus aureus* and *Escherichia coli* and antifungal assay was performed against *Fusarium solani* and *Aspergillus niger* by well diffusion method. The DES extracts showed significant antibacterial and antifungal activity against these strains using Rifampicin and Terbinafine standards, respectively. The vanillic acid, gallic acid, sinapic acid; quercetin, caffeic acid, and cinnamic acid were characterized by HPLC as main bioactive components of *Mentha arvensis* L.

Keywords: Deep eutectic solvent; *Mentha arvensis* L.; Response surface methodology; Phytochemicals; Microwave assisted extraction; ultrasound assisted extraction; Choline chloride.

**P.71. ISOLATION OF PATHOGENIC BACTERIA IN BURN PATIENTS OF
RAWALPINDI**

¹Muhammad Muzammal Saeed, Muhammad Shaheez Ahmad and Muhammad Javaid Asad

¹Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: muzammalsaeed143@gmail.com

Burn wounds are the most sensitive wounds in order to microbial attack. They are directly exposed to microbial spores and can infect by pathogens. These pathogens cause pus over the wounds and inhibit their healing as long as these microbial cures are not done. To cure these microbes it is necessary to identify them so that their antibiotic medications can be performed. The purpose of my study will be isolation of these pathogens to identify their class and order. In initial step I will collect the samples by rubbing swab on the pus wounds of the patients with proper care and transfer them to the Microbial-Laboratory. Then culturing of these samples will perform over the Selective media and Nutritive media for 18-24 hr. at 37 °C. Then the morphological study of these cultural colonies, with the help of senior microbiologists, will be performed. More over Gram Stains and other Chemical tests (i.e. Oxidase and Catalase test) will be conducted to identify the specimen. API's and other Biochemical test will also perform where these are necessary. At the end Results will be recorded and Statistical values will be taken for further analysis of their prevalence. This study will be helpful in order to check the current pathogenic attacks in the burn cases and conductance of their prevention measures.

P.72. COMPARISON OF SERUM RESISTIN LEVELS IN OBESE AND NON-OBESE PATIENTS OF POLYCYSTIC OVARIAN SYNDROME

Zara Hussain^{*,1}, Mateen Abbas², Abdul Muqeet Khan³ and Muhammad Ahsan Iqbal⁴

^{1,2}Quality Operations Laboratory (QOL), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

⁴University of Health Sciences, Lahore.

Corresponding author Email: mateen.abbas@uvas.edu.pk

Polycystic ovary syndrome (PCOS) is a heterogenous syndrome and is the most common cause of anovulation in adult women. Resistin hormone is a contributing factor in the development of insulin resistance (IR) and obesity. In this study resistin levels were calculated in three groups, obese PCOS patients, non-obese PCOS diagnosed patients and control (healthy lean) group. Fifty three samples of diagnosed patients were collected for each group and resistin levels were measured by ELIZA. Serum resistin concentrations were found elevated in both PCOS patients subgroups. Mean serum resistin concentration was elevated in women with PCOS as compare to the control group (Mean $\pm$ SD) (21.32 ± 2.82 vs 8.93 ± 0.97). In obese PCOS patients serum resistin levels were elevated (Mean $\pm$ SD) (23.19 ± 2.51) as compare to non-obese PCOS patients (Mean $\pm$ SD) (19.45 ± 1.64). This data shows that increased serum resistin secretion in obese PCOS women may play an important role in causing ovarian hyperandrogenism.

Keywords: Polycystic ovary syndrome, resistin, health, ELIZA.

**P.73. DETECTION OF ORGANOPHOSPHATE PESTICIDE RESIDUES IN
TOMATO COLLECTED FROM MARDAN DISTRICT, KPK**

Babar Naseem^{*,1}, Mateen Abbas², Abdul Muqet Khan³ and Zara Hussain⁴

Quality Operations Laboratory (QOL), University of Veterinary and Animal Sciences
(UVAS), Lahore, Pakistan

Corresponding author Email: mateen.abbas@uvas.edu.pk

The use of pesticides in modern agricultural production helps to increase yields and improve the quality of products. However, their inadequate usage leads to accumulation of their residues in the agricultural products. Organophosphates are the most extensively used insecticides in many crops due to its low persistency and high killing efficiency. The purpose of this study was to examine the organophosphate pesticide residues (Diazinon, Malathion, Chlorpyrifos, Chlorfenvinphos) in tomato. A total number of 150 tomato samples were randomly collected from different Tehsils of Mardan District, KPK for detection of pesticide residues. High Performance Liquid Chromatographic (HPLC) method was used for analysis after extraction with ethyl acetate. Tomato samples from Tensile Mardan contained both Malathion and Chlorfenvinphos and tomato samples collected from Takht Bhai contained only Malathion. While in Tehsil Katlang, we did not find any sample having organophosphate residues. On an average samples collected from Mardan district contains 41% Malathion, 25% Diazinon, 23% Chlorfenvinphos, and 17% chlopyrifos. The results indicated that residues of organophosphate pesticides are existing largely in tomato fields and its level (>MRL) might be toxic to human health.

Keywords: Pesticides, organophosphate, tomato, HPLC.

P.74. QUANTITATIVE DETERMINATION OF PYRETHROID RESIDUES IN TOMATO COLLECTED FROM KHYBER PAKHTUNKHWA

Abdul Muqeet Khan^{*,1}, Mateen Abbas², and Zara Hussain³ Babar Naseem⁴

Quality Operations Laboratory (QOL), University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

Corresponding author Email: mateen.abbas@uvas.edu.pk

This study was aimed to check the residue of frequently used Pyrethroid pesticides including Cypermethrin, Deltamethrin, Bifenthrin, and Permethrin in tomato taken randomly from retail market of Khyber Pakhtunkhwa (Nowshera, Mardan, Swabi, Swat, Shangla). A simple isocratic High Performance Liquid Chromatographic (HPLC) method with Diode array detector was used to quantify the residual level of pesticides in tomato after liquid-liquid extraction using ethyl acetate. About 84% of the collected samples were found contaminated with pesticides. Cypermethrin was found with the highest mean concentration level of 17.02 mg/kg (ranged from 1.6-60.21 mg/kg) followed by Bifenthrin with mean residue level of 2.83 mg/kg (ranged from 0.59-9.21 mg/kg). The mean residue level of Permethrin and Deltamethrin was 2.30 mg/kg (0.48-8.16mg/kg) and 1.57 mg/kg (0.43-7.05mg/kg), respectively. The results also indicated that 80%, 73%, 67% and 53% of the tomato samples were contaminated with Permethrin, Cypermethrin, Bifenthrin, and Deltamethrin, respectively.

Keywords: Pyrethroids, pesticides, residue level, Tomato

**P.75. DENDRITIC CELLS: PROMISING CANDIDATES FOR IMMUNITY IN
AVIAN COCCIDIOSIS**

Zeshan Zulfikar*, Shahid Ur Rehman, Sidra Bashir, Muhammad Khalid Bashir, Umar

Farooq, Muhammad Ashraf, Muhammad Farooq Khalid

University of Agriculture Faisalabad, Sub Campus Toba Tek Singh

Centre for Applied Molecular Biology & Forensic Sciences University of the Punjab

Corresponding author Email: zeshan@gmail.com

Dendritic cells are key regulator of immune response with capability of provoking both innate immunity and specific acquired immunity. Initiation and maintenance of immune response by DC are channeled through chemical and mechanical signals. It activates T cell immune response by forming a synapse with T cells strings. It gains attention in vaccination procedure due to remarkable property of having as “Nature’s adjuvants”. Livestock animals like chicken and turkey are majorly effected by intestinal disease Coccidiosis caused by *Eimeria* spp, which causes loss to economy due to low production rate of poultry. To overcome all these losses, vaccination procedures with DC have been adopted, for sustainable immune response. DC derived vaccines might be a useful tool to overcome avian diseases. Vaccinating the chickens with DC derived exosomes having parasite antigens is an auspicious tool to overcome avian Coccidiosis. These DC derived exosome have been proved valuable strategy for developing vaccines against numerous pathogens. It might be convenient in near future to develop vaccines from viruses, bacteria and parasites through antigen loaded DC derived exosomes. These DC derived vaccine will have all the features of live vaccines and also avoid the intrinsic risk. Vaccines consisting of exosomes will both preserve all the positive aspects of live parasite vaccines and avoid their inherent risks. Therefore, Immunity created through exosomes could be a better alternative in case of conventional methods of disease control in that case when anticoccidial drug regulation is tightened.

Keywords: Dendritic cells, immunity, exosome

P.76. POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD AND FOOD MATERIALS-A REVIEW

Hira Zikar¹, Zahid Mahmood², Muhammed Shahid¹, M. Muneeb Abbas³

¹Department of Biochemistry, University of Agriculture, Faisalabad.

²Department of Biochemistry, Government College University, Faisalabad.

³Department of Applied Chemistry, Government College University, Faisalabad.

Corresponding author Email: hirazikar19@gmail.com

Polycyclic aromatic hydrocarbons are organic compounds produced by the incomplete combustion of organic material. Present in different kind of foods vegetable, fruits, coffee and smoked meat and fish. Concentrated in human bodies by these food. In general smoked food contains the large amount of polycyclic aromatic hydrocarbons. These compounds having the adverse health effect causing the damage of DNA known as carcinogenic and mutagenic. Different techniques used for the determination of PAH: synchronous fluorescence spectroscopy (SFS), gas chromatography coupled with time-of-flight mass spectrometry (GC-TOF-MS), flow injection-capillary electrophoresis, bioassays and capillary electrophoresis, high-performance liquid chromatography (HPLC) with UV and/or fluorescence detection. HPLC is considered an ideal technique for determining the PAH.

**P.77. IMPACT OF POSTHARVEST VAPOUR HEAT TREATMENT ON
BIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF MANGO**

Muhammad Suliman Shah¹, Aman Ullah Malik¹, Mahmood Ul Hasan¹, Ahmad Sattar Khan¹,
and Muhammad Shahid

¹Postharvest Research and Training Centre, Institute of Horticultural Sciences, University of
Agriculture, Faisalabad.

³Department of Biochemistry, University of Agriculture, Faisalabad.

Corresponding author Email: ss.shahgee11@yahoo.com

Mango is the prime fruit crop cultivated around the globe due to pleasing taste, flavor and amazing aroma. Fruit quality considered as major factor involves during the whole supply chain. However, fruit fly is a serious quarantine pest which downgrade fruit quality and effect its export value. Various techniques are being used as quarantine treatment (hot water treatment, vapour heat treatment, irradiations) before export. Present study was conducted to evaluate the effect of vapour heat treatment on external and internal quality attributes of mango. Selected fruits were exposed to vapour heat treatment for 25 min at 47 °C temperature. Treated (VHT) and non-treated (washed) fruits were assessed for biochemical parameters; (total soluble solid (TSS), titratable acidity (TA %), TSS:TA ratio, vitamin C), and phytochemicals (total phenolic contents, antioxidants). Most of the above parameters such as total soluble solid (TSS), titratable acidity (TA %), TSS/TA ratio, ascorbic acid content, total phenolic content (TPC) and antioxidants (DPPH%) showed statistically non-significant results among VHT treated and non-treated fruits. Hence, conclusively non-significant results depicted that vapour heat treatment had no negative effect on quality of fruit which can satisfy consumer demand and market standards.

**P.78. OPTIMIZATION AND CHARACTERIZATION OF PLEUROTUS ERYNGII
LACCASE IMMOBILIZED ON CHITOSAN BEADS**

Sadia Aslam, Muhammad Asgher, Zinayyera Subhani, Tanzila Sahar

Department of Biochemistry, Government College Women University, Faisalabad, Pakistan.

Department of Biochemistry, University of Agriculture, Faisalabad

Enzymes are the most versatile and robust biological catalysts having more competitive applications in industrial processes as compared to the chemical catalysts. A large number of enzyme catalyzed reactions have been utilized and commercialized for the production of valuable products and variety of strategies have been adopted like screening of enzymes from natural sources, immobilization and random mutations in order to get enzyme with great stability, specificity and catalytic efficiency to exploit their biological potential. Production of laccase enzyme by using white rot fungi through solid state fermentation of lignocellulosic biomass 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 eryngii* 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 70% was found using 1% glutaraldehyde solution after six hours of incubation. Kinetic parameters, K_M and V_{max} values, were determined respectively as 114 μ M and 370 U/mL for free enzyme at pH 3 and temperature 40°C. While K_M and V_{max} values were 103 μ M and 380 U/mL for immobilized laccase at pH 9 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 dye aqueous solution. The laccase immobilized on chitosan beads was very effective for removal of textile dyes solution which creates an important environmental problem.

Keywords: Optimization, Immobilization, Solid State Fermentation, *Pleurotus eryngii*

P.79. EVALUATION OF AGRO INDUSTRIAL WASTES AGAINST OXIDATIVE STRESS

^{a*} Farkhanda Arshad, ^a Razia noreen, ^b Huma ambreen & ^a Iqra aslam

^a Government College University Faisalabad,

^b Government College Women University Faisalabad,

Corresponding author Email: ^{a*} Farkhandaarshad77@gmail.com

Obesity is a societal dilemma universally which results in increased body weight and unnecessary fat accumulation in the body. Consumption of antioxidants offers health benefits including protection against metabolic diseases. The objective of study is to examine the antioxidative potential of dried peel extracts against oxidative stress in obese person and Bio-evaluation of peels in obese human subjects against oxidative stress. Dried fruits peel powder was analyzed for polyphenolic contents using HPLC. Obese subjects were divided into two groups (one treatment and other as placebo). The blood samples were taken at baseline and at the end of study period and were analyzed for glutathione peroxidase, malondialdehyde and TBARS using respective methods. For disease safety evaluation, CBC, LFT, RFT and lipid profile tests were also performed. Complete randomized design was used for statistical analysis. Bioefficacy study of peel extract against obese persons shows promising results in terms of reduction of obesity through eradication of oxidative stress, lowering level of cholesterol, glucose level, serum lipid profile, LDL levels and increased antioxidant status of patient.

Keywords: HPLC: High performance liquid chromatography, TBARS: thiobarbituric acid reactive substances, CBC: Complete blood count, LFT: liver function test, RFT: renal function test, LDL: low density lipoprotein

P.80. ENHANCED NITROGEN USE EFFICIENCY BY USING DIFFERENT UREASE AND NITRIFICATION INHIBITORS COMBINATION IN WHEAT CROP

^aZill-e-Huma, ^aNazish Jahan and ^bKhalil-Ur-Rehman

^aDepartment of Chemistry, University of Agriculture, Faisalabad, Pakistan

^bDepartment of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: zilehuma098@gmail.com

Nitrogen plays a vital role in crop productivity, regarding both its economic and ecological aspects. A continuous supply of Nitrogen is necessary for the healthy growth of plant since nitrogen deficiency can cause slow growth. The excessive fertilizer application for good crop production is harmful for environment especially for ground water due to leaching of excess nitrates (NO_3^-) and also cause economic burden on farmers. So, there is the need to improve fertilizer nitrogen use efficiency to reduce nitrogen loss in the form of NO_3^- , N_2O and NH_3 . The main objective of this study was to enhance N use efficiency by using different combinations of urease and nitrification inhibitors in wheat crop. A lysimeter experiment was conducted on wheat crop in soil of three districts Faisalabad, Gujranwala and Sheikhupura during Dec 2017-Feb 2018. According to Randomized Complete Block Design (RCBD) eleven different treatments were applied in wheat crop: first treatment only Urea was apply as a control at rate of 4.52gN/40Kg and ten treatments: Urea + Urease and NI inhibitors combination (A,B,C,D,E,F,G,H,I,J) (0.1%, 0.25%, 0.5% conc.) (n=3) were applied in each district. For estimation of NO_3^- loss leachate samples collected after treatments were applied at specific time of interval at 2, 14 and 28 days. NO_3^- were estimated by colorimetric technique: chromotropic acid method. The results showed that combination A reduce the NO_3^- loss by 58.8%, 40% and 46% in three districts. Combination F also decrease the nitrate loss by 53.76%, 29.87% and 44.56% in Faisalabad, Gujranwala and Sheikhupura respectively. Overall results suggested that urea applied with urease and nitrification inhibitors has the potential to reduce nitrates loss by leaching significantly ($p>0.05$). Urease and nitrification inhibitors have the potential to reduce the nitrate loss through leaching, improve nitrogen use efficiency due to retention of nitrogen in soil for longer time period and make wheat cropping more environment friendly.

Keywords: Nitrates loss, Urea, Urease, Nitrification inhibitors, wheat and rice crop

P.81. EFFECT OF DIFFERENT LEVELS OF *MORINGA OLEIFERA* LEAF MEAL ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS OF BROILER CHICKS

Faiza Mukhtar and Aftab Ahmad

¹Department of Zoology, University of Agriculture, Faisalabad

Corresponding author Email: fairfaizafsd@gmail.com

The study was conducted to investigate the effects of different levels of *Moringa oleifera* leaf meal on growth performance and carcass characteristics of chicks. The research project was performed at Poultry Farm during the month of March and April 2018. In performance parameters weekly body weight, weekly feed consumption and feed conversion ratio were recorded. For carcass characteristics live weight, dressed weight, thigh meat and breast meat yield, drumstick weight and other organs weight etc. Two hundred day old chicks indiscriminately dispersed into four groups of forty broiler chicks. This experiments will be further allotted in to five replicates comprising ten chicks to each. Four experiment ratios were prepared. Ratio A contain 0% MOLM whereas ratio B, C and D contain 3%, 5% and 7% MOLM respectively. At the end of the experiment, blood sample from 2 birds were selected and analyzed for the determination of liver function tests i.e. AST, ALT and ALP. At the last day of experiment, two birds from each pen were slaughtered to record the organ weight. Sensory evaluation was done to check juiciness, tenderness, flavor and color. The data were analyzed by using factorial under completely randomized design and their means were compared by Tukey's test. Analysis of variance of the data revealed organ weight and sensory evaluation was found to be non-significantly different. Results of AST, ALT, and ALP showed significant. Based upon the results, broilers given the feed having MOLM showed better performance to other.

Keywords: Broiler chicks, Performance parameter, Carcass characteristics, organ weight, liver function test.

P.82. IMPROVE NITROGEN USE EFFICIENCY BY USING 2,5-DICHLOROANILINE AND AMMONIUM THIOSULPHATE AS NITRIFICATION INHIBITORS

^aZeeshan Khadim, ^aNazish Jahan, ^bKhalil-Ur-Rehman, ^aZill-e-Huma and ^aMuhammad Asif

^aDepartment of Chemistry, ^bDepartment of biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: mianxeeshan2018@gmail.com

A large portion of nitrogen chemical fertilizers applied (for increasing crops yield and quality) is losing every year in the form of nitrate leaching, N₂O and NH₃. These N losses can be decreased by using different inhibition technologies, nitrification inhibitors inhibit is one of the technologies used to suppress the activity of soil nitrifying bacteria and they increase the possibilities of retention of NH₄⁺ form of nitrogen in the soil. The present study was conducted to evaluate the nitrification inhibitor potential of two nitrification inhibitors, ammonium thiosulphate and 2,5-dichloroaniline on wheat and rice crop. This study will be carried out in two phases, in first phase the physio-chemical properties of the soil was analyzed and in the second phase the nitrification inhibition potential was evaluated on rice and wheat crop grown season of 2017-2018. The three treatments of each inhibitor were applied with conc. of T₁: 0.1%, T₂: 0.25% and T₃: 0.5% (w/w). The urea was applied as control at a rate of 0.782gN/6kg soil (120 kg N/ha). The whole experiment was design through statistical model RCBD (Randomized complete block design) with three replicates. Methods like, Kjeldhal, Spectrophotometry and Flame photometry were used. The average values of different physio-chemical parameters were obtained after analysis of 15 soil samples selected from the Sheikhpura District were as follows; pH (7.86±0.06), N (41.7±0.003mg/ 1g of soil), P (131.2±0.006mg/ 5g soil), soil moisture contents (0.96±0.09g/ 10g soil), EC (593±0.07mS/cm), K (123±0.017mg/ 10g soil) and soil organic matter (4.56±0.01%). The results obtained for nitrates estimation showed that in rice crop the T₂: 0.25% and T₃: 0.5% treatments of ammonium thiosulphate and also treatment T₁:0.1% of 2,5-dichloroaniline showed significant reduction in NO₃⁻-N losses. For wheat crop, T₁: 0.1% and T₂: 0.25% of ammonium thiosulphate and T₁:0.1% and T₃: 0.5% of 2,5-dichloroaniline was showed that these treatments showed effective inhibition potential. It was concluded that all the nitrification inhibitors showed effective results in minimizing nitrogen losses in the form of nitrates into the ground water and the physio-chemical properties have also played an important role in nitrogen losses from soil through various mechanisms.

Keywords: ammonium thiosulphate, 2,5-dichloroaniline, nitrates losses, nitrification inhibitors

P.83. ASSESSMENT OF LIPID PRODUCTION POTENTIAL OF OLEAGINOUS YEAST ISOLATED FROM SALINE HABITAT

Muhammad Arif Nazir^a, Rizwan Aslam^{a, #}, Dr. Saima Shahzad Mirza^b, Sultan Ali^a, Ghazanfar Abbas^a, Muhammad Ashraf^a, Hira Jawaid^c, Asma Andleeb^d.

^aInstitute of Microbiology, ^bPunjab Bioenergy Institute, PARS, ^dDepartment of Zoology, University of Agriculture, Faisalabad

c: Department of Zoology, Punjab University, Lahore

Corresponding author Email: rizwanaslam@uf.edu.pk

With the increase in worldwide population, the consumption of fuel is also increasing day by day. We mainly depend on the non-renewable resources of energy, which have become short in their reservoirs. So, it is need of the hour to switch our dependence from non-renewable resources to renewable resources, to save them from diminishing. The main renewable source of energy is biodiesel for which lipids are precursors and obtained from oleaginous yeast. But these are unable to meet the required requirements. So, we have to adopt some reasonable methods to make this more productive, faster and cost effective. In my study, different strains of oleaginous yeast were isolated from saline soil and analyzed for their productivity for lipids. Those species were identified by using different microscopic, biochemical techniques and their lipid production potential were analyzed on different carbon sources. Glucose and Xylose were two carbon sources used as main carbon source to grow oleaginous yeast. Total seven strains were isolated among them two strains efficiently used xylose for carbon source 0.98658 ± 0.026 and 0.98994 ± 0.019 , and produce dry mass 4.378 ± 0.010 and 5.269 ± 0.010 , to produce lipid 0.811 ± 0.041 and 0.91 ± 0.108 as output. While four strains produces dry cell masses 3.278 ± 0.009 , 6.698 ± 0.22 , 2.251 ± 0.004 and 2.543 ± 0.0132 to give lipid contents 1.029 ± 0.065 , 0.993 ± 0.025 , 1.132 ± 0.226 and 1.071 ± 0.137 on the expense of 0.90552 ± 0.01 , 0.91287 ± 0.15 , 0.63924 ± 0.09 and 0.74844 ± 0.19 respectively. We can produce these lipids at industrial scale and then use them as precursor for the biodiesel production.

**P.84. USE OF MEDICINAL PLANT EXTRACTS FOR INHIBITION OF
AFLATOXINS PRODUCING FUNGUS AND SCREENING OF ANTIFUNGAL
PEPTIDES BY SDS-PAGE**

Maliha Nawaz, Farah Kanwal, Muhammad Umar, Sarfaraz Hussain, Fozia Saleem.

Department of CABB, University of Agriculture Faisalabad

Corresponding author Email: fozia@ualberta.ca

Aflatoxins are carcinogenic poisons produced by certain molds. Among those *Aspergillus parasiticus* and *Aspergillus flavus* are major fungi causing major food spoilage. Contaminated food when processed, aflatoxin enter in general food supply where they have set up in both human and pet foods, including feed stock for the agricultural animals. In developing countries almost 4.5 billion people are lethally exposed to uncontrolled amounts of aflatoxins. An effective and less costly method is used to avoid risk and adverse effects on human health. So, natural medicinal plant extracts are being used to inhibit aflatoxins producing fungus and detoxification of aflatoxins. The present study intended to determine antifungal activity of 5 herbal plants and screening of antifungal peptides using SDS PAGE analysis. The anti- fungal activity of these five medicinal plants were examined against *A. flavus* and *A. parasiticus* growth. Significant inhibition was attained from all five medicinal plants extracts at three different concentrations especially at 0.7% concentration. We found that clove and lemon grass showed 100% inhibition against both *A. flavus* and *A. parasiticus* while garlic did not inhibit *A. Flavus* but showed 75% inhibition of *Aspergillus parasiticus* till seventh day of experiment. We also performed gel overlay assay to study the antifungal peptides in these five medicinal plants. It was observed in SDS GEL under the antifungal peptides less or no growth of fungus was found. The analysis of this experiment showed significant results. Thus, these five medicinal plant extracts may successfully use to replace synthetic pesticides and provide an alternative method to staple food from toxic fungal contamination.

P.85. METABOLITE PROFILING OF HEALTHY AND HUANGLONGBING (HLB) INFECTED CITRUS LEAVES.

Muhammad Umar¹, Zohaib Hassan², Fozia Saleem³

^{1,2,3} Department of CABB, University of Agriculture Faisalabad.

Corresponding author Email: fozia@ualberta.ca

Citrus is very important crop in Pakistan, particularly in Punjab (95% area of cultivation) because of favorable temperature and environmental conditions. Pakistan is at 12th position in citrus production in the world. Citrus Huanglongbing (HLB) has emerged as biggest threat to the citrus industry of all over the world including Pakistan. Hence it is the major cause of citrus decline in Pakistan. Citrus greening is caused by vector transferrin species of *Candidatus Liberibacter* bacterium: including *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter americanus* and *Candidatus Liberibacter africanus*. The bacterium is transmitted from one plant to other by psyllid vector. Early detection of HLB is seen as potential long term management strategy in controlling HLB. Currently, PCR is the only technology approved for regulatory use for detection of infection but the limitation of PCR is, it cannot detect the infected bacteria at the early stage. An infected tree can take up to a year to test positive for the bacterium with traditional PCR testing methods. There are many efforts going on to develop the methods for the detection of this disease in initial stages. Considering, Metabolomics has offered relevant insights into discovery of biomarkers “metabolite fingerprint” that is specific to the causal pathogen. We used high performance liquid chromatography (HPLC) for metabolite profiling of healthy and HLB-infected leaves of citrus. We identify more than 25 metabolite that are highly differentiating diseased plant from healthy ones. This study helped us in the development of new diagnostic methods for the diagnosis of HLB at its early stage.

Keywords: Metabolites, Biomarker, Citrus greening

P.86. PREPARATION OF APPLE PEEL NANOSUPENSION AND EVALUATION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORY POTENTIAL

¹Moiffa Gohar, ^{1*}Nazish Jahan, ²Khalil-ur-Rahman and ¹Syeeda Iram Touqeer

¹Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

²Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: nazishjahanuaf@yahoo.com

Nano suspension is considered as one of the promising techniques to enhance therapeutic effect of less bioavailable drugs. It is estimated that apple peel provides approximately 22% of dietary phenolics like flavonoids and phenolic acids. The problem associated with these compounds is their low bioavailability, which leads to reduce their applications in drug delivery system. Apple peel nanosuspensions were prepared in the presence of different stabilizers (PVA, SLS, PVP, HPMC, Tween 80 and PEG 400) by antisolvent precipitation technique to overcome the bioavailability issues. PVA was selected as best stabilizer for the nanosuspension of apple peel on the bases of characterization studies. Central composite design of response surface methodology was used for the optimization of nanosuspension. Optimal nanosuspension showed 130.5nm particle size and -10.5 mV zeta potential value. *In vitro* Angiotensin Converting Enzyme Inhibitory potential, antimicrobial, antioxidant and hemolytic activities were evaluated. Apple peel nanosuspension exhibited potent antioxidant, angiotensin converting enzyme inhibitory potential and safe towards erythrocytes. Crude extract and its nanosuspension were unable to show resistance against *B. subtilis* and *E. coli*.

Keywords: Angiotensin Converting Enzyme, Apple peel, Nanosuspension, Response surface methodology

**P.87. MOLECULAR DETECTION OF METHICILLIN (MECA) AND
VANCOMYCIN RESISTANT (VANA) STAPHYLOCOCCUS AUREUS FROM
DIGESTIVE TRACT OF APIS MELLIFERA**

Ammara Mushtaq^a, Rizwan Aslam^{a,*}, Muhammad Ashraf^a, Sultan Ali^a, Ghazanfar Abbas^a.

^aInstitute of Microbiology, University of Agriculture, Faisalabad

Corresponding author Email: rizwanaslam@uf.edu.pk

Honey is traditionally used as medicine for treatment of various microbial infections. *Staphylococcus aureus* is gram positive cocci. It is a normal resident of skin and mucus membrane. It is a main cause of food born infections. About 241,000 morbidity and 5000 mortality rates occurred by *S. aureus* in United States. The pathogenicity of *S. aureus* is due to different virulence factors and adhesive proteins. *S. aureus* showed resistance against different antibiotics including kenamycin, penicillin, vancomycin and methicillin. Objectives: The purpose of my research was to detect the genetic profile of different antibiotic resistance genes of *S. aureus* and check their prevalence in *Apis mellifera*. Methodology: Total 35 isolates were collected from digestive tract of honey bee (*Apis mellifera*) and futhure dissected. The rectum and midgut of honey bee were cultured in Tryptone Soya Broth (TSB). Samples were cultured on blood agar and further subcultured on Mannitol Salt Agar medium. Identification of the microbes was done by using microscopic examination and biochemical testing. Kirby Bauer Susceptibility method was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines to check the resistance pattern of antibiotics for confirmed positive isolates. PCR and agarose gel electrophoresis techniques were used for analyzing the resistant genes of *S. aureus*. Results: Out of 35 isolates, 33 isolates showed positive results for *S. aureus*. By Kirby test, methicillin was 80% resistance than other antibiotics. DNA of positive isolates was extracted by Phenol Chloroform Method. 33 isolates were analyzed for amplification of *mecA* and *vanA* genes by PCR technique. After that, PCR product was run on 1.5% agarose gel by agarose gel electrophoresis method. Results were visualized by Gel Documentation system which showed that 25(75%) isolates were *mecA* resistant with 533bp bands and 5(15%) strains were *vanA* resistant with 1032bp bands. Conclusion: It is concluded that methicillin (*mecA* gene) has high prevalence than vancomycin (*vanA*) in digestive track of honey bee (*Apis mellifera*).

**P.88. CALCIUM DEPENDENT PROTEIN KINASE RELATED KINASE (CRK1)
REGULATED SALT STRESS RESPONSE IN ARABIDOPSIS.**

Khush Bakhat Afzal^{*a}, Aftab Ahmad^{a,b}, Muhammad Zubair Ghouri^c, Sultan Habibullah Khan^{b,c} and Zulqurnain Khan^d

^aCotton biotechnology lab, US-Pakistan Center for Advanced Studies, ^bDepartment of Biochemistry, ^cCenter for Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad

^dInstitute of Plant Breeding and Biotechnology, Muhammad Nawaz Sharif University of Agriculture, Multan.

Corresponding author Email: aftab.ahmad@uaf.edu.pk

For many signaling pathways, calcium is the center and used as the second messenger. The level of calcium concentration depends on the stimuli as NaCl stress. In Arabidopsis, many stress conditions cause the increased level of the calcium in cytosol detected by the calcium sensors that convert the calcium signals into response. Previously, we have reported that in one of our mutants, *stc8*, calcium-dependent protein kinase related kinase 8 (CRK1:At2g41140) was activated both in control and 150mM NaCl stress level. In Arabidopsis, 34 CDPKs (calcium-dependent protein kinases) and 8 CRKs (CDPKs related kinases) are present to activate the many pathways to tolerate ionic and osmotic imbalance in stress conditions and exhibit the specific development effects. CDPKs act as rate limiting factor for sensitivity against salt stress. T-DNA knock-out (KO) lines for AtCRK1 were sensitive to NaCl. In addition, over-expression (OE) lines were tolerant to NaCl compared with wild-type plants. We also performed microarray for both KO and OE plants to identify the genes regulated by AtCRK1. The results showed that AtCRK1 play an important role under salt stress in Arabidopsis.

**P.89. INVESTIGATION OF *FELINE PANLEUKOPENIA VIRUS* ANTIBODIES IN
DIARRHEIC CAPTIVE FELINES IN DISTRICT FAISALABAD, PAKISTAN**

Farrah Deeba^{1*}, Anas Sarwar Qureshi², Muhammad Haleem Tayyab¹, Naureen Rana³,
Ayesha Anwar³, Khurram Ashfaq¹

¹Department of Clinical Medicine and Surgery, ²Department of Anatomy,

³Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad

Corresponding author Email: farrah.deeba@uaf.edu.pk

Feline infectious enteritis due to Feline panleukopenia virus (FPV) is a highly fatal disease of domesticated and wild felines with worldwide distribution. The present study was devised to establish prevalence of antibodies for feline panleukopenia virus in different breeds of domesticated cats in district Faisalabad. In 2016-17, blood samples were drawn from 50 cats with history of enteritis from all areas of district Faisalabad for detection of antibodies to FPV infection. All the sera harvested by centrifugation were subjected to hemagglutination inhibition assay (HIA). Out of 50 samples tested, 37 samples were found HIA positive for FPV infection with overall 74% prevalence in cats in district Faisalabad. Multivariable analysis revealed significant association ($p < 0.05$) of seroprevalence of FPV with juvenile age group (<1year), lack of vaccination and enteritis history, while breeds and sex were considered as non-significant factors with $p > 0.05$. This study concluded higher prevalence of FPV antibodies in domestic cats of district Faisalabad and suggested proper vaccination protocol to avoid fatal outbreaks in household cats.

Keywords: Panleukopenia, Hemagglutination assay, Enteritis, Felines, Seroprevalence, Faisalabad

P.90. IN VITRO ANTIBACTERIAL POTENTIAL OF SELECTED MEDICINAL PLANTS AGAINST SOME GRAM NEGATIVE AND GRAM POSITIVE BACTERIA

Javaria Hafeez^{1*} and Fatma Hussain¹

¹ Clinico-Medical Biochemistry Laboratory, Department of Biochemistry,

University of Agriculture, Faisalabad-38040, Pakistan.

Corresponding author Email: javaria.hafeez@yahoo.com

Plants are rich source of various medicines as they produce numerous bioactive molecules which might be an alternative source to antibiotics to cure antibiotic-resistant bacteria. In the current study, in vitro antibacterial potential of five medicinal plants such as *Momordica charantia* L. (bitter melon or bitter gourd), *Syzygium cumini* L. (jamun), *Allium sativum* L. (garlic), *Zingiber officinale* L. (ginger) and *Parthenium hysterophorus* L. (carrot grass) were assessed by using different solvent extracts such as methanol, chloroform, ethanol, n-butanol, ethyl acetate, n-hexane and aqueous. These extracts were investigated against two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two gram-negative (*Escherichia coli* and *Pasteurella multocida*) bacteria by using agar well diffusion method. Rifampicin was used as standard antibiotic. Some of the fractions of the selected medicinal plants showed greater activity against some tested bacteria than the standard antibiotic used. Maximum activity against the *Escherichia coli* was shown by the methanolic extract of *S. cumini* as compare to *Momordica charantia*, *A. sativum*, *Zingiber officinale* and *Parthenium hysterophorus*. n-hexane extract of *Z. officinale* showed bacteriostatic activity against all tested bacterial strain except *Staphylococcus aureus*. It is suggested that these selected medicinal plants are potent to be used as pharmaceutical drugs but additional studies may be required to reveal more definite information about medicinal plants and their active components.

Key words: Gram-positive bacteria, gram-negative bacteria, agar well diffusion, rifampicin

**P.91. HETEROLOGOUS EXPRESSION OF ARCHAEAL
FRUCTOSYLTRANSFERASE GENE**

Komal Ghauri¹, Nayla Munawar^{2,*}, Hazrat Ali¹, M. Afzal Ghauri¹ and Munir A. Anwar¹

¹ Industrial Biotechnology Division, National Institute for Biotechnology & Genetic Engineering, Faisalabad.

² Department of Biochemistry, University of Agriculture, Faisalabad.

Fructose polysaccharides, called fructans, are gaining tremendous importance due to their beneficial effects on human and animal health as prebiotics. The use of prebiotics in animal/poultry feed for modulation of the gut microbial equilibrium is important for alleviating the use of antibiotics. However, the gastrointestinal microflora consist of approximately 10¹⁴ colony forming units (cfu)/g of various types of both harmful and beneficial bacteria. It is not clear, which fructans are the most suitable substrates for the selective growth of specific beneficial species or strains. Therefore, it is direly needed to explore diverse range of living systems to acquire variety of unique prebiotic compounds. Several archaeal strains are annotated to harbour fructan synthesis (fructosyltransferase: ftf) genes in their genome sequences (www.cazy.org). The indication of the occurrence of the genes coding for GH68 enzymes in archaeal strains is obtained only from metagenomic data and no studies have been done on the characterization of these genes, which might have role in the synthesis of novel prebiotic compounds. The archaeal strains, namely *Halalkalicoccus jeotgali* and *Halomicrobium mukohataei*, have shown that the growing cells of these archaea do not synthesize fructans despite they harbour the ftf genes. Modelling of the FTF proteins of these archaea using bioinformatics tool has shown that their catalytic residues are perfectly superimposable with the bacterial FTF enzymes, which synthesize fructans. Therefore, expression of these archaeal proteins in heterologous system in order to study their structure-function relationship is intended. In this context, ftf gene from archaeon *Halalkalicoccus jeotgali* for expression in *E. coli* and *Pichia pastoris* system has been successfully cloned.

P.92. HINDRENCES AND SCOPE OF VACCINE DEVELOPMENT IN PARASITIC DISEASES

Muhammad Usman Naseer^{1,2*}, Zia ud Din Sindhu¹, Muhammad Imran¹

¹Department of Parasitology, University of Agriculture, Faisalabad-38040, Pakistan

²Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL-61802, USA

Corresponding author Email: usmanshani717@gmail.com, munaseer@illinois.edu

Until now, chemotherapeutic drugs have shown predominance over vaccines for the prevention and control of parasitic diseases of livestock. Although, today's market has continuous flow of vaccines but this trend is discouraging in anti-parasitic vaccines. It is a fact that chemotherapeutic drugs were being developed much earlier than vaccines, there are multiple factors that hinder the development of vaccines against parasitic diseases. Shelf life, storage and transportation and production and development costs are some of the vital factors for commercial viability of vaccines. Current commercial drugs have 100% efficacies which might be the biggest factor to develop a vaccine in the presence of these drugs. In this scenario, this will never be an easy job to convince a user that vaccine with less than 100% efficacy is beneficial as compared to other commercial drugs. By far, most of the economic losses by parasites are sub-clinical, while these anti-parasitic drugs are mostly used to treat the clinically sick animals and to maximize the profits. These practices are expected to be threatened in the coming future due to increased awareness about emergence of drug resistant parasites. Consequently, there is an increasing trend to reduce the extensive use of commercial drugs and to find some reasonable alternatives of disease prevention where vaccines can play a pivotal role. So it can be reasonably assumed that more parasitic vaccines will find their way to control the parasitic diseases in near future.

Keywords: Vaccines, Drugs, Parasites, Efficacy

P.93. GENOTYPE MARKERS ASSOCIATED WITH RESISTANCE TO PARASITISM IN GOATS

Muhammad Imran^{1*}, Muhammad Sohail Sajid¹, Muhammad Usman Naseer^{1,2}, Asim
Shamim³ and Fatima Yousaf⁴

¹Department of Parasitology, ⁴Department of Biochemistry, University of Agriculture,
Faisalabad-38040, Pakistan,

²Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL-61802,
USA,

³Department of Pathobiology, University of Poonch Rawalakot, Azad Jammu and Kashmir, ,
Corresponding author Email: imran.asghar@uaf.edu.pk

Parasitism particularly gastrointestinal (gi) nematodes remains a major constraint associated with the breeding of goats (*capra hircus*) under grazing/browsing conditions. The increasing development of anthelmintic resistances in endo parasites and community concern on the use of chemicals in farm industry requires novel and more sustainable methods. These solutions included the improvement of the host response against worms rely either on the genetic selection of lines or breeds of hosts or on the manipulation of nutrition to increase host resistance and/or resilience and lastly grazing management. Principally, this resistance of the host is measured through expression of phenotypic and genotypic markers. In this prospect, a research project has successfully been completed in the department of parasitology, university of agriculture faisalabad with the aim to evaluate indigenous goat breeds (beetal, teddy, dera din panah, nachhi) for their natural resistance towards gi parasitism through phenotypic markers. This response was measured both in natural parasitic and artificial infection challenge. Furthermore, exploring some genotype markers associated with breed resistance to parasites is promised during next phase of project. These markers include major histocompatibility complex (mhc), ovine leukocyte antigen, ovine mhc class ii antigens, interferon gamma (ifng) gene, quantitative trait loci (qtl) mapping, microsatellite markers, single nucleotide polymorphisms (snps) and illumina ovine bead chips. This theme of writing is to disseminate knowledge about selective breeding of parasite resistant goat breeds through expression of phenotypic and genotypic markers. This practice will not allow the establishment of disease and consequently may reduce the use of anthelmintics. Also, this will ensure availability of chemical free meat and milk for human consumption.

Keywords: genotypic markers, parasite resistance, gastrointestinal nematodes, goat breeds, Pakistan

P.94. ARTEMISIA SCOPARIA AN UNEXPLORED SECRET OF NATURE

Toseef Fatima^{1*}, Khalid M. Khan¹, Khalil-ur-Rahman¹, Nazish Jahan² and Asma Yaqoob¹

Department of Biochemistry, Department of Chemistry², University of Agriculture,

Faisalabad. 38000. Pakistan

Corresponding author Email: Toseef_Fatima27@yahoo.com

Artemisia scoparia belongs to a diversified and taxonomically difficult *Genus Artemisia* which includes more than 500 species with long history in folk medicines due to the presence of sundry phytochemicals. Meta-analysis of different biochemical analysis from the past 15 year research reveals diverse nature of many species of *Genus Artemisia*, but biphasic nature of *Artemisia- scoparia* for Artemisinin contents from vegetative to reproductive stages still demanding attention of researchers. Through this platform I want to highlight some unexplored aspects of *Artemisia scoparia* to underpin the phenomena behind that diverse property. So that researcher can find a better natural substitute for NSAIDS in the form of Artimisinin with lesser side effects.

Keywords: *Artemisia Scoparia*, Folk medicine, Artimisinin, NSAIDS

**P.95. THE PHYSIOCHEMICAL COMPOSITION OF DATE PALM FRUITS AT
THREE DIFFERENT EDIBLE STAGES**

Muhammad Waseem^{*a}, Summar Abbas Naqvi^a, Muhammad Jafar Jaskani^a, Muhammad
Shahid^b Hina Fatima^b, Iqar Ahmad Khan^a

^aPomology Laboratory, Institute of Horticultural Sciences, University of Agriculture,
Faisalabad 38040, Pakistan; ^bProtein Molecular Biology Laboratory, Department of
Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: wasimm45@gmail.com

The fruit of date palm (*Phoenix dactylifera* L.) has rich source of nutrition for humans as it provides a large amount of sugars, minerals, fibers, vitamins, phytonutrients, fatty acids, protein and amino acids. The objective of the present study was to investigate the effect of maturity on the physiochemical composition of date palm fruits. Fifteen varieties were taken from KPK and their physicochemical analysis were done at three different maturity stages (khalal, rutab and tamar). The analysis fruits revealed that the antioxidant activity (65-90 %), total phenolic contents (420.28 to 1502.73 mg GAE/100g, FW), total flavonoids (19.67 to 77.12 mg CE/100g, FW), ascorbic acid (21.80 to 57.41 mg/100, FW), total anthocyanins (0.10 to 0.52 mg/100g, FW), total carotenoids (62.3 to 91.25 mg β -carotene equivalent/100g, FW), antioxidant enzymes (SOD, POD and CAT) and the soluble protein contents (5.73 to 2.75 g/100g) were higher in higher at khalal stage but decreased at fully ripened (tamar) stage. Moreover, total sugar contents (35.47 to 48.17 mg/100g, FW), reducing sugars (19.98 to 32.85 mg/100g, FW) and minerals (nitrogen, phosphorus and potash) were lower at khalal stage and increased from rutab to tamar stage. It was found that the non-reducing sugars were present only at khalal and rutab stage. Gulistan, Haq Nawaz, Basra and Dhaki cultivars revealed best overall values in examined compounds. Our results revealed that variation in different biochemical attributes is mainly depended on the difference in fruit maturity stage and cultivar. Date palm cultivation can be expended by selecting cultivars exhibiting high values of beneficial biochemical attributes.

P.96. IN VIVO INVESTIGATION OF CEFTAZIDIME INDUCED OXIDATIVE STRESS IN LIVER KIDNEY AND GILLS OF *LABEO ROHITA*

Abubakar Siddique¹ and Kashif Jilani²

¹Department of Zoology and Fisheries

²Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Ceftazidime is an antibiotic drug, clinically used for the cure of many gram positive and gram negative bacterial infections. It belongs to third generation cephalosporin. *Labeo rohita* is an omnivorous, silver colored fresh water fish. Commonly, it is found in slow flowing, weedy or standing waters. It feeds mainly on zooplanktons and phytoplanktons. Oxidative effect in kidney, liver and gills of *Labeo rohita* was examined with two physiological doses (low 250mg/20L and high 500mg/20L) of ceftazidime. Increased generation ROS by some exogenous and endogenous sources, results in an imbalance state, called oxidative stress. It causes oxidation of major bio molecules and leads to several degenerative disorders like ischemia, mutagenesis and chronic inflammation. Drug exposure duration was consists of five days. Phsiochemical parameters of water were measured on daily bases. After five days exposure, fish were dissected and organs separated. Samples were prepared for enzyme assay to measure the activity of antioxidant enzymes. Oxidative stress was measured by activity of anti-oxidant enzymes comprises of superoxide dismutase, glutathione peroxidase and catalas. Higher activities of antioxidant enzymes revealed the conclusion that Ceftazidime caused oxidative stress in fish organs. Elevated values of catalase in organs became the proof of ROS production in organs exposed to drug. For correlation and analysis one way ANOVA with Tukey's test was applied.

Keywords: ceftazidime, oxidation, inflammation

**P.97. COMPARISON OF SYNTHETIC YIELD OF BIODIESEL THROUGH
PHOTOCATALYTIC AND CATALYTIC PROCESSES USING CU DOPED LIME
COATED OVER METAL SHEET**

Sehrish Naz ^a, Ijaz Ahmad Bhatti ^a, Maryam Yousaf ^a, Hafiza Wajiha Umer Farooq ^a,
Muhammad Mohsin ^a, Muhammad Tahir Hussain ^b

^a Department of Chemistry, University of Agriculture, Faisalabad

^b Department of Applied Sciences, National Textile University, Faisalabad

Corresponding author Email: sehrishnaz1576@gmail.com

Biodiesel is biodegradable, environment friendly and an efficient substitute of petroleum diesel fuel. It reduces the emission of greenhouse gases in the environment and can be economically prepared by using waste cooking oil (WCO) through transesterification process by using an efficient photocatalyst. In current study we have designed Cu/lime/metal hybrid (solar photocatalyst) to enhance the yield of biodiesel production from waste cooking oil. Spherical shaped and highly crystalline hexagonal structure of Cu/lime nanoparticles were efficiently synthesized as clear Scanning Electron Microscopy (SEM) and X-ray Diffraction analysis (XRD). Furthermore, the presence of Oxygen, Calcium, and Copper in Energy Dispersive X-ray Spectroscopy (EDX) confirmed doping and purity of Cu/lime. Later on, photocatalytic transesterification reaction for biodiesel production from waste cooking oil was carried out under sunlight using Cu/lime/metal hybrid. All operational parameters such as Methanol to oil ratio, reaction temperature and time for the biodiesel synthesis were optimized by Response Surface Methodology. The highest yield percentage of the photocatalytic transesterification reaction was 86% having reaction conditions 6:2 methanol to oil ratio at 50°C for 3 hours. Evaluation of amount of synthetic biodiesel was done by Gas chromatography analysis. Gas chromatography analysis confirmed the presence of fatty acid methyl ester from which following are Methanoic acid, Butyric acid and Capric acid having peak area 2.3, 0.2, 15.6 respectively. Biodiesel produced from WCO was found to contain high octane number 55, low iodine value 58, saponification value within the range of 232-258 and Acid value less than 1. The cloud point ranges from 3°-5°C. Synthesized biodiesel can be used as a substitute to petroleum diesel without any modification in engine.

**P.98. PHYTOCHEMICAL SCREENING & PHARMACOLOGICAL EVALUATION
OF *SENEGALIA MODESTA* (WALL) P. J. H. HURTER**

Sana Gul*¹, Hidayat Ullah Khan¹, Nisar Khan²

¹Department of Chemistry, ²Department of Botany,
University of Science and Technology, Bannu (28100), KP, Pakistan.

In present study, shed dried bark of *Senegalia modesta* (WALL) P. J. H. Hurter Bark was extracted with 70% ethanol. Qualitative Phytochemical analysis of extract of plant bark showed the presence of flavonoid, tannins, saponins, glycosides, cumarins, terpenoids, anthraquinones and phlobatannins. The amount of total phenol content 2500 mg/ 100 g GAE was recorded and total flavonoid content 0.4 g /0.5 g was found in extract dry weight. The crude extract showed effective antioxidant results with IC₅₀ values of 49.56±9.68% at 100 µg / ml and 74.34±1.23 % at 250 µg / ml for H₂O₂. The α-amylase maximum % inhibition of the ethanolic extract was 96.01±0.94 at 4 mg/ml. The crude extract showed highest Zone of Inhibition at 10 mg/ml extract concentration against *Shigella dysentery* 12mm, 60%, *Staphylococcus aureus* 12mm, 60%, *Escherichia coli* 12mm, 60% and *Klebsiella pneumonia* 13mm (65%). The extract showed a decrease in blood glucose levels at 100 mg / kg body weight is about 56.81 % and at 200 mg / kg body weight is about 68.11% and the standard drug i.e. 2 mg glibenclamide / kg body weight decrease about 63.48 %. The extract showed an increase in body weight of diabetic rats about 1.55% at 100 mg / kg body weight and 1.4% at 200 mg / kg body weight while the standard drug at 2 mg/ kg of body weight cause increase in body weight of diabetic rats about 1.01%. Liver marker enzymes (Alanine transaminase, alkaline phosphatase) levels were restored with extract about 40±19.38 µL ALT and 175.2±0.79 µL ALP at 100 mg / kg body weight and at 200 mg / kg body weight was about 20±28.32 µL ALT and 128±21.9 µL ALP. The extract at concentration of 100 mg / kg body weight reduced triglycerides 75±8.45 mg / dl, LDL cholesterol 41±0.24 mg / dl, high HDL cholesterol 49±1.57 mg / dl and at 200 mg / kg body weight reduced triglycerides 65.19±12.88 mg / dL, LDL cholesterol 27±6.05 mg / dl and high HDL cholesterol 60.6±3.60 mg / dL, respectively. The serum levels of urea 31±0.23 mg / dl and creatinine 0.74±0.17 mg / dl were restored by post-treating with the bark extract at 100 mg / kg body weight and the extract at 200 mg / kg body weight returned serum urea 26±2.46 mg / dl and creatinine 0.7±0.19 mg / dl compared with control group. The results shows that the extract of plant bark possess potent antioxidant, phytotoxic antibacterial and anti-diabetic effects both in-vitro and in-vivo which might be due to the presence of bio-active compounds.

**P.99. EVALUATION OF CEPHRIDINE TRIGGERED OXIDATIVE STRESS IN
LIVER KIDNEY AND GILLS OF *CIRRHINUS MRIGALA***

Humaira Sharif¹ and Kashif Jilani²

¹Department of Zoology and Fisheries, ²Department of Biochemistry,

University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Cephridine is an antibiotic drug that belongs to first generation cephalosporins. It is significantly active against broad range of gram negative bacteria as well as gram positive. It is an acid stable drug that is used for the treatment of various infections. *Cirrhinus mrigala* is a herbivorous fish that feeds mainly on decaying vegetation. It is a freshwater fish inhabiting in freshwater bodies and its common habitats include river, ponds, canals haors, beels ditches, flood plains and lakes. Oxidative stress is the disturbance of cellular metabolism due to the imbalance between the production of reactive oxygen species and antioxidants. It causes the oxidation of bio molecules and leads to many degenerative diseases. In this study the physiological doses of cephridine were used to estimate the oxidative effects due to the generation different oxidants in the extracts of selected organs like kidney, liver and gills of *Cirrhinus mrigala*. Oxidative stress triggered by cephradine was confirmed in all isolated organs as antioxidants enzymes SOD, CAT and GPx activities were significantly goes down in comparison to with control group. Tukey's tests with one way ANOVA were used for statistical analysis.

Keywords: Cephridine, oxidative stress, Liver

**P.100. DETERMINATION OF MOXIFLOXACIN INDUCED OXIDATIVE STRESS
IN LIVER KIDNEY AND GILLS OF *CYPRINUSCARPIO***

Nabila Akbar¹ and Kashif Jilani²

¹Department of Zoology and Fisheries

²Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Moxifloxacin is an antibiotic drug, belongs to fourth generation cephalosporins. Specifically it is used against gram-positive bacterial infections like pneumonia, conjunctivitis, bronchitis, sinusitis and abdominal infections. *Cyprinus carpio* is a fresh water omnivorous fish, can be recognized by large shiny scales. It is placed in teleost family. It is locally adapted in the world and cultivated on global range. Oxidative effect in liver, kidney and gills of *Cyprinus carpio* was examined after treatment with physiological doses of moxifloxacin. Increased production of ROS by endogenous, exogenous sources, leads to an imbalance state, called oxidative stress. It causes oxidation of major bio molecules and leads to several degenerative disorders like ischemia, mutagenesis and chronic inflammation. Drug exposure duration was of five days. Physiochemical parameters of water were measured on daily bases. After five days exposure of drug, fish were dissected and organs separated. Samples were prepared for enzyme assay to evaluate the activity of antioxidant enzymes. Antioxidant enzymes like SOD, CAT, GPx are helpful to measure the oxidative stress. Higher activities of antioxidant enzymes revealed the conclusion that moxifloxacin caused oxidative stress in fish organs. Elevated values of catalase, superoxide dismutase and glutathione peroxidase in organs became the proof of ROS production in organs exposed to drug. One way ANOVA with Tukey's was applied for statistical analysis.

Keywords: Moxifloxacin, SOD, ANOVA

P.101. IMMUNOMODULATORY ACTION OF NEEM (*Azadirachta indica*) IN NEWCASTLE DISEASE INFECTED BROILER CHICKS

Shazia Nusrat, Farzana Rizvi, Shah Nawaz*, Ayesha Ramzan
and Muhammad Shahzad Shafiq

Department of Pathology, Faculty of Veterinary Science, University of Agriculture,
Faisalabad

Corresponding author Email: malikshahnawaz786@gmail.com

Leaves of neem (*Azadirachta indica*) were used as immunomodulatory agents in commercial broiler chicks experimentally infected with Newcastle disease virus. A total of 175, day-old healthy broiler chicks were divided into five groups at random; 35 chicks in each group. One group was kept as control with no neem supplementation. The chicks of rest of groups were given feed supplemented with neem leaves powder @ 8 g/kg of feed from 3 to 42 days of trial. On 30th day of age, the chicks were challenged with NDV by sub cutaneous route. Cellular immune response was checked with Tuberculin test and phagocytic index was determined by carbon clearance assay while Humoral immune response was estimated against sheep RBCs and NDV. Serum antibody production against NDV was determined with haemagglutination inhibition (HI) test. Results of the trial showed that body weight, organ weight (thymus, bursa, spleen & lungs) were significantly higher in neem supplemented groups as compared with control group. Humoral & cellular immune response was significantly increased in neem supplemented groups as compared with control group. Antibody titer was increased from 2.1 to 7.92 in neem supplemented group challenged with NDV. Whilst antibody titer against sheep red blood cells was increased from 4.6 to 5.8 in control and SRBC supplemented group respectively. Microscopically, congestion and hemorrhages were observed in spleen of NDV infected birds. There was hypocellularity of lymphocytes of spleen and bursa of Fabricius of infected birds. Sever congestion and alveolar dilation was observed in lungs of NDV infected birds. No gross and microscopic changes were seen in visceral organs of NDV infected plus Neem supplemented broiler chicks. From this study it could be concluded that Leaves of neem (*Azadirachta indica*) supplementation in feed can act as immunomodulatory agent especially during viral infection.

P.102. PREVALENCE OF FATTY LIVER IN OBESITY AND IN LIVER DISEASED PATIENT

Fatima Ilyas¹, Sadia Sana² and Naheed Akhter²

². College of Allied Health Professionals, Directorate of Medical Sciences.

Govt. College University, Faisalabad.

Corresponding author Email: sadiasana203@gcuf.edu.pk

Objective: The purpose of this study was to evaluate the prevalence of fatty liver in obese and other liver disease patients. **Introduction:** Fatty liver is a disease in which there is an accumulation of excess fat in the liver. This accumulation of fat is caused without alcohol consumption and called as NAFLD (non-alcoholic fatty liver). The non -alcohol fatty liver is frequently occurring liver disease and the occurrence of fatty liver is increasing around the world. It develops from simple form of fatty liver to NASH, which further result in fibrosis, cirrhosis and eventually lead to organ failure if it is not treated. NAFLD developed frequently in people who are suffering from obesity, hepatitis, diabetes and metabolic disorder which may result in cardiovascular diseases. There are different factors which are associated with obesity such as age, unhealthy diet, lifestyle, high intake of fats. **Methodology:** 80 patients with fatty liver were selected. This cross-sectional study was carried out in Allied hospital Faisalabad from July 2018-November 2018. Patient's BMI calculated which provide the guide to obesity level. Abdominal ultrasound is used to evaluate the size, shape and echogenicity of the liver. **Results:** Out of 80 patients 27 were male and 53 were female. The mean age and liver size were 45 ±20 and 15 ±3 respectively. About 62% patients were obese and overweight while 37% patients were suffering from hepatitis or have hepatitis C in past. Hepatitis C is most commonly occurring as compared to hepatitis B in NAFLD. **Conclusion:** NAFLD is co-existing with hepatitis patient but with less prevalence than obesity. Patients suffering from NAFLD have higher BMI.

Keywords: BMI (body mass index), NAFLD (Non-alcoholic fatty liver disease), NASH (Non-alcoholic steatohepatitis), Obesity.

**P.103. EVALUATION OF AGRO INDUSTRIAL WASTES AGAINST
OXIDATIVE STRESS**

^{a*} Farkhanda Arshad, ^a Razia noreen, ^b Huma ambreen & ^c Iqra Aslam

^{a,c} Government College University Faisalabad,

^b Government College Women University Faisalabad

Corresponding author Email: Farkhandaarshad77@gmail.com

Obesity is a societal dilemma universally which results in increased body weight and unnecessary fat accumulation in the body. Consumption of antioxidants offers health benefits including protection against metabolic diseases. The objective of study is to examine the antioxidative potential of dried peel extracts against oxidative stress in obese person and Bio-evaluation of peels in obese human subjects against oxidative stress. Dried fruits peel powder was analyzed for polyphenolic contents using HPLC. Obese subjects were divided into two groups (one treatment and other as placebo). The blood samples were taken at baseline and at the end of study period and were analyzed for glutathione peroxidase, malondialdehyde and TBARS using respective methods. For disease safety evaluation, CBC, LFT, RFT and lipid profile tests were also performed. Complete randomized design was used for statistical analysis. Bioefficacy study of peel extract against obese persons shows promising results in terms of reduction of obesity through eradication of oxidative stress, lowering level of cholesterol, glucose level, serum lipid profile, LDL levels and increased antioxidant status of patient.

Keywords: High performance liquid chromatography, Thiobarbituric acid reactive substances, Complete blood count, liver function test, renal function test, low density lipoprotein

P.104. EVALUATION OF THE BIOACTIVE ROLE OF INDEGENOUS VIOLA ODORATA LEAF EXTRACTS

Tehreem Sikander, Aqsa Anum Bhatti and Zahid Mushtaq*

Bioactive Molecules Research Lab (BMRL), Dept of Biochemistry,

University of Agriculture, Faisalabad. 38040

Corresponding author Email: zahidmushtaquaf@uaf.edu.pk

Medicinal plants are rich source of bioactive compounds and other secondary metabolites and used to prepare drugs for preventing infectious diseases because natural sources like plants have least or no side effects and less expensive as compared to other synthetic drugs. In the present study bioactive extracts from *Viola odorata* leaves using organic solvents from more non-polar to polar solvents like methanol (CME), *n*-hexane (*n*-HF), chloroform (CF), ethyl acetate (EAF) and water were extracted. The residue left was further solubilized in methanol (MSF) and water (WSF). The bioactive potential of leaves extracts was checked by using different assays for evaluating antimicrobial, anti-oxidant, thrombolytic, cytotoxic, α -amylase inhibition, lipase inhibition potential whereas phytochemical screening including Total phenolic compounds (TPC) and Total flavonoid compounds (TFC) was also done. Antioxidant potential was evaluated by using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay. The maximum antibacterial activity was shown by MSF (methanol soluble fraction) 14 mm against *E.coli*. Antifungal activity was shown on *Fusarium solani* strain only in which *n*-HF gave maximum ZOI of 30.33 mm. The maximum TPC was shown by MSF 3.4 mg/g and maximum TFC was shown in *n*-HF 0.21 mg/g. MSF exhibited maximum α -amylase inhibition $42.163^A \pm 2.74$. CME exhibited maximum lipase inhibition $95^A \pm 0.89$. EAF showed maximum antioxidant activity that is $62.13^A \pm 4.26$. CF showed maximum cytotoxicity ($76.31^A \pm 0.252$). Maximum clot lysis was shown by MSF $26.44^A \pm 0.485$. Moreover HPLC results showed high amount of phenolics in CME and *n*-HF that could be related to their bioactivities. Hence *V. odorata* proved an excellent source of bioactive compounds that can be analyzed individually in future.

Keywords: *Viola odorata*, antimicrobial activity, cytotoxicity, enzyme inhibition

P.105. DETERMINATION OF ANTI-ERYPTOTIC POTENTIAL OF *PIPER NIGRUM* L. (BLACK PEPPER) PLANT EXTRACTS IN CIPROFLOXACIN TREATED ERYTHROCYTES

Kashif Shabbir and Kashif Jilani

Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Programmed death of erythrocytes is also known as eryptosis which may leads to anemia. *Piper nigrum* also known as ‘black pepper’ is a medicinal plant which show antioxidant activity and reduce the effects produced due to oxidative stress in the body. Ciprofloxacin a well-known antibiotic inhibits DNA gyrase which is essential for bacterial DNA replication. In current study, the effect of ciprofloxacin was evaluated in the induction of oxidative stress which may be the possible mechanism of eryptosis. Similarly anti-eryptotic potential of *Piper nigrum* extracts was determined. During first phase of study, erythrocytes samples were exposed to various concentrations of ciprofloxacin in ringer solution for 48 hours and oxidative stress was measured through antioxidant enzyme activity. Induction of eryptosis and role of calcium in its stimulation was measured by mean cell volume as well as the phenomenon of hemolysis was determined. In second phase, anti-oxidative and anti-eryptotic outcome of *Piper nigrum* through aqueous and ethanolic extracts were observed in ciprofloxacin treated erythrocytes. Results reflects that ciprofloxacin stimulate oxidative stress by reducing the level of antioxidant enzymes with superoxide dismutase, glutathione peroxidase and catalase. Similarly promotes eryptosis and hemolysis. Anti-eryptotic potential of ethanolic and aqueous extracts of *Piper nigrum* were confirmed and compared. Both ethanolic and aqueous extracts of *Piper nigrum* have very strong anti-oxidative and anti-eryptotic potential with 20 mg/ml and 40 mg/ml concentrations. While during comparative observation of both extracts the aqueous extract has much more potential against eryptosis with 40 mg/ml concentration as compared to ethanolic extract with same concentration.

Keywords: Eryptosis, Ciprofloxacin, hemolysis

P.106. EVALUATION OF ACTINOMYCIN D INDUCED ERYPTOSIS AND ANTI ERYPTOTIC ACTIVITY OF *WITHANIA SOMNIFERA* AND *CAMELLIA SINESIS* EXTRACTS

Mehroze Javed and Kashif Jilani

Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Programmed red blood cells fatality termed as the phenomenon of eryptosis was distinguished with the prominent factors of phosphatidylserine displacement at the red blood cells membrane site, cell membrane blebbing and depreciation of the erythrocytic cell. This suicidal death of erythrocytes was mainly stimulated by oxidative stress, osmotic shock, energy deficiency and kinase action. Actinomycin D is a cytotoxic drug. It is known inhibitor of RNA production and inducer of the programmed death of the nucleated cells. *Withania somnifera* and *Camellia sinesis* commonly known as ‘ashwaganda’ and “green tea” were medicinal plants with reported antioxidant potential. Role of actinomycin D determined in eryptosis by treating RBCs with different concentrations of the drug. As a possible mechanism of eryptosis, spectrophotometric analysis was done to determine the oxidative stress. The antieryptotic as well as antioxidative potential of both these herbal plants manifested against the oxidative potential of the anticancerous drug actinomycin D. The induction of eryptosis was confirmed by cell size measurement. Anti-oxidative mechanism of *Withania somnifera* and *Camellia Sinesis* evaluated by incubating actinomycin D treated red blood cells with various concentrations of aqueous and ethanolic extracts of used plants. Statistical analysis made using one way ANOVA with Tukey’s test as post-test.

Keywords: actinomycin D, eryptosis, ANOVA

P.107. POTENTIAL OF *MANGIFERA INDICA* SEEDS AND ITS COMPOSITES FOR THE REMOVAL OF DIRECT YELLOW-50 AND REACTIVE GREEN-5 FROM AQUEOUS MEDIA

Asma Jabeen^a and Haq Nawaz Bhatti^b

^a Department of Chemistry, University of Agriculture Faisalabad, Pakistan

^b Department of Chemistry, University of Agriculture Faisalabad, Pakistan

Corresponding author Email: asmajabeen7861@gmail.com

Adsorption is the most promising and robust technology for the purification of water from toxic pollutants. Pollutants such as dyes are directly discharged into water stream from pigment manufacturing, painting, photographic, cosmetic, tanning, dyeing and textile industries without proper treatment. The present research work was designed to use the *Mangifera indica* (mango) seeds and its composites for the removal of dyes Direct yellow (DY-50) and Reactive Green (RG-5). Native biomass (NB), biomass magnetic (BM) and biomass alginate (BA) composites were prepared for this adsorption study. Batch study shows that the optimum pH for DY-50 was observed in acidic range with BM composites and neutral with NB and BA composites. Similarly removal of RG-5 was observed in acidic range with all composites. On increasing the contact time for both dyes biosorption capacity also increases, maximum removal of DY-50 was obtained after 120 min with NB & BM, and 90 min for B. RG-5 showed maximum adsorption after 120 min for BM & BA and 90 min for NB. Kinetic study for the adsorption phenomenon was carried out by using pseudo-first and second order kinetics. Similarly, Langmuir, Freundlich and Temkin were applied for determine the adsorption characteristics. Thermodynamic parameters were also determined to see the nature and feasibility of adsorption mechanism.

Keywords: Dyes, Composites, Kinetic study, Equilibrium study, Thermodynamics

**P.108. SYNTHESIS AND CHARACTERIZATION OF NANOPARTICLES FROM
EUCALYPTUS CAMALDULENSIS AND THEIR APPLICATION ON COTTON
FABRIC FOR MEDICAL PURPOSES.**

Hira Munir, Asima Mumtaz and Muhammad Talha Zubair*

Department of Biochemistry and Biotechnology

University of Gujrat, Pakistan

The purpose of present study was the synthesis of zinc and silver nitrate nanoparticles from *Eucalyptus Camaldulensis*. To evaluate their antimicrobial potential, they were tested against bacterial and fungal strains. The hemolytic activity was performed to check whether they are toxic or non-toxic. Ames test was carried out to evaluate the mutagenic potential. Their characterization was done through X-Ray diffraction (XRD), thermogravimetric analysis (TGA), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The selected gum was effective against the selected strains. Hemolytic and Ames test reveal that the gum samples were non-toxic and non-mutagenic. The synthesis of nanoparticles was confirmed through characterization. XRD analysis shows that nanoparticles show more crystalline nature as compare to crude and purified gums which confirm the synthesis of nanoparticles and purity of samples. Through, TGA analysis, it was identified that the thermal stability of samples was increased after synthesis of nanoparticles and weight loss not occur rapidly as compare to crude and purify gum. Thus, in future, these prepared nanoparticles on the cotton fabric can be used as suitable substrate for medical products such as antimicrobial textiles and wound dressings.

P.109. STUDY OF BODY MASS INDEX AND MICRONUTRIENTS IN POST-MENOPAUSAL OSTEOPOROTIC WOMEN

Ambreen Awan and Feroza Hamid Wattoo

Department of Biochemistry, PMAS Arid Agriculture University Rawalpindi, Pakistan

Objective: This study was conducted to correlate the Body mass index (BMI) of post-menopausal women with serum level of calcium, phosphorus, sodium, potassium, chloride and total serum protein in their blood. **Methods:** This study was conducted at the PIMS hospital Islamabad and Benazir Bhutto Hospital Rawalpindi from July 2017 to September 2018. For the study, two hundred post-menopausal Osteoporotic women having aged between 45-85 were selected. A food frequency questionnaire (FFQ) was used for the nutritional assessment. Biuret method was used to measure the concentration of total protein in serum. Calorimetric method was used to measure the serum concentration of calcium, phosphate, potassium, chloride and sodium. All the statistical analysis was performed using the SPSS version 21. **Results:** BMI and age were significantly higher in osteoporotic women as compared to control (p-value <0.05). Serum levels of Calcium and potassium were significantly lower (p-value < 0.05) in cases as compared to control while serum levels of phosphorus, sodium and chloride were significantly higher (p-value <0.05) in cases as compared to control. Total serum protein gives the non-significant difference between cases and controls (p-value >0.05). Sodium and chloride gives significant positive correlation with BMI while phosphorus gives significant negative correlation with BMI in osteoporotic patients.

Conclusion: In conclusion, abnormal levels of micronutrients in serum play important role in the development of post-menopausal osteoporosis. Adequate nutrition plays a significant role in the prevention and treatment of osteoporosis.

Keywords: BMI, Micronutrients, Nutrition, Post-menopausal osteoporosis

P.110. PLANT DISEASES DIAGNOSIS AND CHARACTERIZATION THROUGH PCR

Saher Naveed¹, Muhammad Hassan¹, Muhammad Waseem Sarwar¹, Ayesha Younus²,
Muhammad Shah Nawaz ul Rehman¹ and Muhammad Mubin^{1*},

Virology Lab, Center of Agricultural Biochemistry and Biotechnology, University of
Agriculture, Faisalabad, Pakistan; Department of Plant Pathology, University of Agriculture,
Faisalabad; ²Laser matter interaction and Nano-sciences Lab, Department of Physics,
University of Agriculture, Faisalabad Pakistan

[Corresponding author Email: mmubin@uaf.edu.pk](mailto:mmubin@uaf.edu.pk)

Agricultural crops in Pakistan are infected by several fungal, bacterial and viral diseases. Citrus is the major fruit crop in Pakistan where kinnow is the dominant cultivar, accounting for the 80% acreage. Most graft transmissible pathogens of citrus present in Pakistan are transmitted to new plants only by using infected nursery material for propagation. There was no equipped lab in Pakistan dedicated to diagnose well before time that what kind of graft transmissible pathogens (GTPs) are present in our citrus germplasm. Even during experiments our researchers usually were not sure that the germplasm under study is free of GTPs or not and if present, what kind of GTPs were there. To solve above-mentioned problems a citrus diagnostic lab at CABB, University of agriculture, Faisalabad is established for the diagnosis of citrus GTPs. Similarly, another effort was started for cotton leaf curl disease. We have established a complete system of viral purification, infectivity analysis and recombination detection in cotton leaf curl geminiviruses. **Objectives:** This Project has three main objectives: 1) To develop an infrastructure to allow local germplasm in Pakistan to be therapied of graft transmissible pathogens and to develop a protocol to screen the local germplasm for GTP especially citrus, 2) To evaluate the germplasm of citrus and citrus relatives for resistance/ tolerance /susceptibility to citrus greening disease and 3) to apply modern sophisticated techniques like quantitative real time PCR for detection of citrus greening and other citrus pathogens and to obtain information on spread of these pathogens in field plantings under different management conditions.

**P.111. PLANT FLAVONOIDS ARE POTENTIAL INHIBITOR OF NEWCASTLE
DISEASE VIRUS V PROTEIN**

Muhammad Waseem Sarwar¹, Saher Naveed¹, Muhammad Hassan¹, Ayesha Younus²,
Muhammad Shah Nawaz ul Rehman¹ and Muhammad Mubin^{1*},

Virology Lab, Center of Agricultural Biochemistry and Biotechnology, University of
Agriculture, Faisalabad, Pakistan; Department of Plant Pathology, University of Agriculture,
Faisalabad; ²Laser matter interaction and Nano-sciences Lab, Department of Physics,
University of Agriculture, Faisalabad Pakistan

Corresponding author Email: mmubin@uaf.edu.pk

Newcastle disease (ND) cause serious economic loss all over the world. Newcastle disease virus (NDV) is single stranded RNA virus belonging to *Paramyxoviridae* family. The V protein of NDV is a multi-domain protein having role in pathogenicity and in viral replication. In this study plant flavonoids were tested as inhibitors of V protein of NDV. The study was focused on sequence analysis, three-dimensional structural modeling and docking analysis of V protein of NDV. Physicochemical properties and sequence analysis showed that V protein is negatively charged protein, acidic in nature and is not very stable. Three-dimensional model of this protein revealed three helical, eight beta pleated sheets and ten coiled regions. More than 250 flavonoids were used in the docking experiment. Ten flavonoids were obtained as potential inhibitors of V protein on the basis of docking score. Further the interaction analysis showed the atoms involved in each interaction of flavonoid and V protein. The results achieved from this study clearly indicate that flavonoids are potential inhibitors of NDV and can be used as anti-NDV agents.

P.112. NUTRITIONAL COMPARISON OF IRRIGATED AND RAIN FED WHEAT VARIETIES OF PAKISTAN

Naila Niaz, Feroza Hamid Wattoo and Shehla Begum

Department of Zoology/Biology, PMAS Arid Agriculture University Rawalpindi, Pakistan

Corresponding author Email: nailaniaz338@gmail.com

Wheat (*Triticum aestivum* L.) is an important cereal crop of Pakistan and it take up the main position in agricultural policies. The current Study was carried out for nutritional Comparison of irrigated and rainfed wheat varieties In this study three irrigated wheat varieties; Galaxy-13, Millet-011 and N. Sarhad was compared with three rainfed wheat varieties; AAS-2011, Shaker-13 and Pak-13. Moisture, Ash, fiber, fat, protein, carbohydrates and Minerals (Na and K) composition was determined Analysis was done with LSD test at significance level 0.05. The results shows that moisture, Ash, fiber, fat, protein and Carbohydrates contents in both varieties were 8.1%-11%, 2%-4.1%, 1%- 1.6%, 2%-2.8%,9.8%-13% and 69.5%-75% respectively. Sodium and Potassium contents were 1.761mg/100g-2.424mg/100g and 5.80mg/100g-7.04mg/100g This study shows that wheat is rich in, protein , fiber, inorganic content , fat carbohydrates and minerals. It was concluded that wheat is natural source of nutrients which has great potential against many diseases. Diet rich in these nutrients provide protection against many disease caused by malnutrition. were study for nutritional assessment.

Keywords: irrigated· rain fed· minerals ·wheat. nutritio

P.113. miRNA: A MULTIDISCIPLINARY APPROACH FROM DIAGNOSIS TO THERAPY

Zeshan Zulfiqar*, Shahid Ur Rehman, Sidra Bashir, Muhammad Khalid Bashir, Umar Farooq, Umair Mahmood, Marium Zehra, Muhammad Farooq Khalid
University of Agriculture Faisalabad, Sub Campus Toba Tek Singh
Centre for Applied Molecular Biology & Forensic Sciences University of the Punjab
Corresponding author Email: 4zeshan@gmail.com

The tissue-specific expression and conservation of individual sequences make miRNAs as promising biomarker in biological pathways of various multicellular species particularly mammals. miRNAs are basically group of short RNAs of 19-24 nucleotides long which when adhere to RNA-induced silencing (RISC) complex serve as negative regulators for protein synthesis by degrading it or translational repression of mRNA transcriptome. Unlike mRNA, the short length and less degradability of miRNAs prove them to be the potential biomarkers in the identification of forensically important biological fluids such as saliva, blood, vaginal and seminal secretions. This has gained a lot of importance in forensic cases like child abuse, sexual assault, paternity claim, mass disasters. Moreover, they also serve as reliable clinical diagnostic and prognosis marker in various diseases such as cancers, cardiovascular diseases (CVDs) and autoimmune diseases. miRNA profile can be used for the evaluation of tumor genesis and progression. miRNA profile helps us to evaluate whether the subjected miRNA is tumor suppressor or oncogenic. In the field of therapy, dysregulation of miRNA may alter the disease pathway which can revert back the normal gene expression. miRNA in tumor therapy with different delivery method like liposome, polymers, conjugates, exosomes and bacteriophages has been used. Synthetic oligonucleotide downregulation has also been adopted to inhibit the miRNA selectively. For this purpose, several miRNA has been discovered which can efficiently be used as biomarkers, and therapeutics. In next few year's research will further refine and validate the miRNA in diagnosis and many miRNA-based therapeutics can be expected.

Keywords: miRNAs, biomarker, biological pathway

**P.114. REVOLUTION IN NON-VIRAL DELIVERY SYSTEM OF CRISPR
GENOME EDITING TOOL**

Zeshan Zulfiqar*, Shahid Ur Rehman, Sidra Bashir, Muhammad Khalid Bashir,

Umar Farooq, Umair Mahmood, Marium Zehra, Muhammad Farooq Khalid

University of Agriculture Faisalabad, Sub Campus Toba Tek Singh

Centre for Applied Molecular Biology & Forensic Sciences University of the Punjab

Corresponding author Email: 4zeshan@gmail.com

An advance and robust genome editing tool CRISPR (clustered regularly interspaced short palindromic repeat) with highly recognized protein cas9 has gained a lot of attention in the field of biomedical research and therapeutics due to its ease of use and efficient genome editing capability. It has been exploited in many field like cancer and other genetic diseases. Different strategies used for delivery of CRISPR/cas9 including direct plasmid vector delivery via modified virus, via guided RNA and cas9 mRNA complex and via cas9 and single guided RNA complex. Most of the time adenovirus based delivery method is used which has lot of cons. Majorly off target DNA damage to the host DNA. Non-viral delivery system is a challenge to scientist. Recently synthetic gold nanoparticles having conjugated DNA and endosomal disruptive polymer have been used for efficient delivery of CRISPR/cas9 protein in mice model. This non-viral delivery system is easy to synthesize and deliver. This is definitely more advantageous over viral delivery system due to low off target effect and it can be used for vast variety of cell. A recent advancement in this field has been made to overcome the off target effects and precise activation of CRISPR/cas9 system by targeted activation of viral vector containing DNA to be inserted. In this situation delivery system is introduced in an inactive form with magnetic nanoparticles. A range of magnetic field is applied after delivery, which will activate the particle to release the DNA and other containing material. This is safer method because a certain range of magnetic field has no effect on nearby off target cells. These non-viral techniques will surely have revolutionized the genome editing research with improve cell entry and transduction efficiency. Despite of ethical concerns, there has been a lot of research ongoing for direct human genome editing. Recent research conducted on direct human genome editing is embryonic level editing in HIV cases and designer babies. It will surely transform our world in near future.

Keywords: CRISPR, Nanoparticles, non viral delivery system

P.115. EPIDEMIOLOGY OF BLOODSTREAM BACTERIAL INFECTIONS AMONG HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PATIENTS IN PUNJAB PAKISTAN

Zara Sabir¹, Sultan Ali^{1*}, Ghazanfar Abbas¹, Muhammad Ashraf¹, Rizwan Aslam¹,
Umar Khalid¹ and kashaf Yaseen¹.

¹Institute of Microbiology, University of Agriculture, Faisalabad.

Corresponding author Email: sultanali@uaf.edu.pk

Human immune deficiency virus is a RNA retrovirus of the genus Lentivirus which affects the immune system targeting the T helper cells (specifically CD4 cells) which help out to defend against various infections. A person is considered to have an advanced HIV disease known as AIDS, when the CD4+ cell count go down below 200. This condition makes the host vulnerable to severe opportunistic diseases. Bacterial bloodstream infections, among opportunistic infections constitute an important public health problem in such cases. In this study prevalence of bacterial bloodstream infection in HIV infected patients was determined. The study population was from different hospitals in Punjab, Pakistan. A total of 100 blood samples were collected and streaked on MacConkey, Blood and Chocolate agar. Positive culture were subjected to conventional microbial identification methods and biochemical testing was used for differentiation. API 20E was further used to identify members of enterobacteriaceae. Antibiotic susceptibility was determined using Kirby-bauer disc diffusion method. Of 100 samples 29 showed positive growth. Gram positive isolates constitutes 9/29 (31%) and 20 /29 (69%) were from Gram negative genera. Among these isolates, E. coli (11/29), Pseudomonas aeruginosa (4/29), Klebsiella pneumonia (5/29) whereas among gram positive, Staphylococcus aureus (7/29) and Methicillin resistant Staphylococcus aureus MRSA (2/29). Gram negative isolates showed sensitivity towards ceftriaxone, novobiocin and ciprofloxacin whereas they show resistance to gentamicin, bacitracin and levofloxacin. In case of gram positive bacteria, they were found susceptible to novobiocin, ciprofloxacin and chloramphenicol and resistant to methicillin. Results showed that the gram negative bacteria are more prevalent in HIV infected patients compared to gram positive bacteria. Molecular characterization of these isolates is required in further studies.

**P.116. SELENIUM N-HETEROCYCLIC CARBENE (SE-NHC) ADDUCTS AS
POTENT CHEMOTHERAPEUTIC AGENTS**

Amna Kamal¹, Muhammad Adnan Iqbal^{1,3,†}, Haq Nawaz Bhatti^{1,*}, Aqsa Habib¹

¹Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

² Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains
Malaysia, Minden, 11800-Pulau Penang, Malaysia

³Organometallic and Coordination Chemistry Laboratory, University of Agriculture
Faisalabad-38040, Pakistan

Corresponding author Email: adnan.iqbal@uaf.edu.pk

The rich diversity of coordination chemistry provides exciting prospects for the design of novel therapeutic agents with inimitable mechanisms of action. Unique physico-chemical features of organometallic compounds have announced them as promising candidates for medicinal purposes and potential alternative drugs for cancer therapy. In the current study, synthetically important and novel benzimidazolium salts and their respective selenium adducts were synthesized and characterized by various spectroscopic techniques (FT-IR and NMR ¹H, ¹³C). Synthesized compounds were tested in vitro against a panel of Cancer Cell lines using MTT assay and the results were compared with standard drug 5-Fluorouracil. All the tested compounds showed a dose-dependent cytotoxic activity against all the selected cell lines. Several Se-NHC compounds and benzimidazolium salts showed better anticancer potential. On the average, pronounced activity of NHC-salts compared to respective Se-NHC adducts might be due to their more lipophilicity

Keywords: Selenium, cancer, benzimidazole, lipophilicity

**P.117. PRODUCTION AND CHARACTERIZATION OF BIODIESEL FROM
SPIROGYRA ELONGATA ALGAE**

Aasma Saeed, Muhammad Asif Hanif and Muhammad Waqar Azeem

Department of Chemistry, Faculty of Sciences,

University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

Species of different plant seeds and micro algae are waste product and having major portion of oil. Biodiesel can be formed by the conversion of these oils. The sample of *Spirogyra elongata* algae was collected from Horticultural area of University of Agriculture Faisalabad. The oil was extracted from *Spirogyra elongata* algae and processed by acid, base and zeolite catalyzed processes to produce biodiesel by the chemical reaction of alcohol and extracted oil. Different physio-chemical parameters like Iodine value, Saponification value, total fatty acid contents etc. were evaluated to characterize the biodiesel from *Spirogyra elongata* algae. The analysis of the biodiesel produced was done by GC-MS. The experiments were conducted in triplicate and data was analyzed by using one way ANOVA.

Keywords: Iodine value, Saponification value, Total fatty acid contents, Biodiesel

**P.118. UTILIZATION OF COMPLEX COMPOUNDS AS CATALYST FOR
BIODIESEL PRODUCTION**

Abeer Aslam, Muhammad Asif Hanif and Rasheed Ahmad Khera

Department of Chemistry, Faculty of Sciences,

University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

Biofuels are alternative and reliable substitutes to fossil fuels due to which the demand of biofuel increases tremendously. However, these are in limited use due to cost factor so there is a need to lessen the production cost with eco-friendly conditions. Up to 15-20% cost of biodiesel production is due to catalysts with aim to reduce the production cost the present study was designed to investigate the biodiesel production from non-edible seed oil by using complex compounds as catalyst. The complex compounds of tin, copper and nickel was used as catalysts and their effect on biodiesel yield was evaluated. For the determination of different physio-chemical parameters like cetane number, pour point, cloud point, density, saponification value, acid value and iodine value different standard assays (ASTM and EN) were used. The compositional analysis of the produced biodiesel was done using gas chromatographic mass spectrometer (GC-MS). For the statistical analysis of biodiesel ANOVA with post-hoc Tukey HSD test was used.

Keywords: Biodiesel, cetane number, pour point, cloud point, density, saponification value

P.119. RECTIFICATION OF NATURAL FRAGRANCES IN FRANKINCENSE OIL

Adeesha Beig, Muhammad Asif Hanif, Haq Nawaz Bhatti and Muhammad Zahid

Department of Chemistry, Faculty of Sciences,

University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

Essential oil which is extracted from different plants is a concentrated hydrophobic liquid contain aroma compounds. The present investigation was designed to extract the essential oil from frankincense resin. Frankincense was purchased from the local market of Faisalabad. Frankincense essential oil contained different constituents that act as by product of frankincense. The essential oils from frankincense were isolated by using hydro distillation, a process which was used for separation of oils from plants extract or resins. Fractionation of essential oil was done by vacuum fractional distillation to rectify the natural fragrances of frankincense oil. The technique which was used for the analysis of these fractions is Gas Chromatography mass spectrometry (GC-MS). The isolated fraction of frankincense was derivitized chemically. All the biological activities of frankincense essential oil and its fractions was determined by parameter of antioxidants, antimicrobial and antifungal activities. The results were evaluated statistically by ANOVA and Tuckey test.

Keywords: Frankincense, Resin, Antioxidants, biological activities

**P.120. FABRICATION OF BIO-NANOCOMPOSITES BY UTILIZING BIOMASS
(AGRO WASTE AND AQUATIC WASTE) FOR HMs REMOVAL FROM WASTE
WATER**

Muhammad Adil

Department of Chemistry, University of Agriculture, Faisalabad

Ecological concerns are driving the development of new bio-based, green products. Over the past few decades, the possibility of exploiting natural biomass (agro waste and aquatic waste) as constituents in nanocomposite materials has been exploring and producing encouraging results. In particular, research is being done for the fabrication of bio-nanocomposites using biomass and metal nanocomposites for the adsorption of Heavy Metals (HMs) from industrial waste water. For this purpose, biomass including crop's residue such as cotton husk and rice stalk, biochar such as peanut husks and egg shells and aquatic waste such as fish scales were coupled with metal nanocomposites. The adsorption of HMs present in industrial waste have been tested using these bio-nanocomposites. This study include synthesis methods, formation mechanisms and surface characteristics of bio-nanocomposites, along with the discussions on HMs removal mechanisms and the effects of environmental factors on HMs removal efficiency. Performance of using bio-nanocomposites to remediate real HMs-containing wastewater and issues associated with its process scale-up are also discussed. This review aims to provide useful information to facilitate the development of HMs removal by bio-nanocomposites.

P.121. META QUANTITATIVE TRAIT LOCUS (QTL) ANALYSIS OF FIBER QUALITY AND AGRONOMIC TRAITS IN UPLAND COTTON.

Ayesha Jabbar, Zahid Mustaq*, Muhammad Jamshed*.

*Department of Biochemistry, University of Agriculture Faisalabad.

Cotton is economically an important crop in Pakistan. Breeders are looking for those techniques which may be helpful for increasing the fiber quality with high yield. Upland cotton (*G. hirsutum*) mostly famous for its high yield but low fiber quality, while *G. barbadense* have high fiber quality with low yield specificity. Fiber quality traits have significant impact on yarn production. Fiber quality traits are genetically complex quantitative traits. Various techniques are used to improve genetic architecture of cotton. Meta QTL (Quantitative Trait Locus) Analysis is one of those advanced techniques which combined and compared results of different studies on the basis of common markers. This method helped us to find cluster or hotspot region on chromosomes on the basis of common markers which are useful from agronomic interest. In this study, we used 404 QTLs from 15 different studies on the basis of common markers. We identified 28 hotspot regions on six chromosomes (C) including C6, C7, C13, C18, C16 and C25. Similarly, we detected four QTL cluster regions on C7, and three cluster regions on C6, C13, C16, C18 and C25. These markers will be helpful in cotton breeding programme to make an elite class variety with high yield and excellent fiber quality through marker assisted selection.

Keywords; Upland cotton, RIL population, Genetic map, Fiber quality traits, SSR markers, Meta-QTL analysis, Stable QTL.

P.122. COMPARATIVE STUDY OF PHYTOCHEMICALS AND ANTI-OXIDANT POTENTIALS FROM NATIVE AND GEMMO-MODIFIED CARDIO-PROTECTIVE NUTRITIOUS PLANTS

Hina Aslam^a, Khalil-ur-Rehman^a, Kauser Parveen^c, Nazish Jahan^b, Nusrat Shafiq^c,
Zara Jabeen^a and Habiba Akram^a

^aDepartment of Biochemistry, ^bDepartment of Chemistry,
University of Agriculture Faisalabad

^c Department of Biochemistry, Government College Women University

Corresponding author email: hinaaslam381@gmail.com

Cardiovascular disease (CVD) is the number one cause of deaths throughout the world. Synthetic medicines have adverse side effects on human physiology. Medicinal and curative properties of plants against several ailments have been acknowledged since prehistoric time due to their biological active compounds which help to update and restore the body balance in living systems. Gemmo-modification is a way of herbal formulation in which young freshly emerging parts of plants are exploited. Phytochemical study of plants becomes an interest among pharmaceutical companies for the development of drugs against diseases. This research has been designed to explore the phyto-constituents of *Allium cepa*, *Allium saivum*, *Terminalia arjuna*, *Moringa oleferia* and *Zingiber officinale* qualitatively and quantitatively in native and gemmomodified samples. Gemmo-modified extracts of all selected plants show significantly higher ($p < 0.050$) total phenolic (TPC), total flavonoids (TF), total alkaloids (TA), total ascorbic acid (TAA), total anthocyanin contents (TAC) and total tannins than their native extract samples. Anti-oxidant potential measured by using two assays (DPPH and ABTS) is higher in gemmomodified *Terminalia arjuna* extract and native extract of *Zingiber officinale*. HPLC analysis of gemmomodified *Terminalia arjuna*, *Moringa* and *Zingiber officinale* reveals the presence of vanilic acid, caffeic acid, ferulic acid, sinapi acid and cinamic acid. This comparative study illustrates a step toward development a better, less expensive gemmo-therapeutic herbal formulation against CVDs.

Keywords: Gemmo-modification; Phyto-chemicals; DPPH, ABTS and cardiovascular diseases.

P.123. INVESTIGATION OF HEPATITIS B, PREVALENCE AND ASSOCIATED RISK FACTORS AMONG PREGNANT WOMEN AT TERTIARY CARE HOSPITAL

Hira Manzoor^a, Rizwan Aslam^{a, *}, Muhammad Ashraf^a, Sultan Ali^a, Ghazanfar Abbas^a.

^aInstitute of Microbiology, University of Agriculture, Faisalabad

Corresponding author Email: rizwanaslam@uf.edu.pk

HBV infection is a general public health dilemma for which reliable screening tests present. Pakistan is a developing country with inadequate medical services has high rates of HBsAg carrier. Hepatitis is inflammation of liver tissues. Hepatitis leads to the jaundice, malaise anorexia, Hepatic failure and death. HBV belongs to family Hepadnaviridae. The size of the particle is 42 nm, also denoted as the Dane particle. HBV is transmitted parenterally, sexually and from mother to their infants. According to WHO an estimated 257 million people are living with hepatitis B virus infection. HBV is a common infection in pregnant women with 2.5% transmission risk from mothers to their infants. During pregnancy viral hepatitis is responsible for causing high maternal complications **Objective:** Our aim of study was to find out the HBV prevalence in pregnant women and to investigate the possible risk factors involve in its transmission. **Study design:** It was a Hospital based cross-sectional study. **Setting:** Tertiary care (Allied Hospital), Faisalabad. **Methodology:** Firstly for qualitative analysis a rapid strip test was performed. Then positive cases were further administered towards ELISA. **Results:** A total of 480 patients were screened, 9 were positive for HBV. **Conclusion:** It is concluded from this study that HBV is less common infection in pregnant women. Sexual transmission, blood transfusion and non-sterilized equipment's are among the common risk factors. We have to expand the counteractive action, analysis and treatment endeavors to dispose of the ailment. In addition, training and financial status additionally assume a vital part in the spread of hepatitis

P.124. SYNTHESIS AND CHARACTERIZATION OF AROMATIC ISOCYANATES-BASED POLYURETHANES

Zahra Akram*, Ijaz Ahmad Bhatti, Maryam Yousaf, Nawal Aslam, Misbah
and Muhammad Mohsin.

Department of Chemistry, University of Agriculture, Faisalabad.

Corresponding author Email: ibrahimaq131@gmail.com

Polyurethane is a unique and versatile class of synthetic polymers which has extensive variety of properties and applications. By changing the composition and nature of reactants, their characteristic can be controlled. In the view of these unique properties of polyurethane, the present study has been focused to synthesize chitosan based polyurethane dispersion by using castor oil (polyol), toluene diisocyanates (isocyanate) and dimethyl propanoic acid as an emulsifier in multistep reaction. At the end aqueous solution of chitosan will be added to obtain waterborne polyurethanes dispersion. The synthesized polymer will be characterized by Fourier transform infra-red spectroscopy and Scanning Electron microscopy. To check the biocompatibility of chitosan-based polyurethanes, antimicrobial analysis and blood hemolytic assay will be performed. Polyurethane will be applied on fabric then this treated fabric will be examined for its tensile and tear strength. Experimental data will be examined statistically using standard deviation.

**P.125. COMPLEXING OF MICRONUTRIENTS AND DEVELOPMENT OF SLOW
RELEASE NANO FERTILLIZERS**

Marium Khaliq¹, Muhammad Asif Hanif*,¹ and Zahid Mushtaq²

¹Department of Chemistry, ²Department of Biochemistry,

University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

The deficiency of micronutrients (B, Fe, Zn, Mn, Mo and Cu) in the soil has become a problem of major and global concern in agriculture sector. The direct application of micronutrients to soil does not serve the purpose well, as most of the added active ingredients are leached out due to watering. Thus a constant and sustained supply of micronutrients deserves attention. The current experiment was designed to analyze the effect of complex micronutrients nano fertilizers with different complexing agents (Na₂EDTA, EDTA, FeSO₄, citric acid, proline and aniline) and micronutrients loaded sodium alginate nano carriers on vegetative development and essential oil contents of basil (*Ocimum basilicum*). Following experiment was conducted to study the effects of nano fertilizers with randomized complete block design (RCBD). Four experimental replicates of each treatment were used. Reasonably, controlled moisture (50%) and temperature (25±2°C) was applied on all plants in greenhouse. ANOVA and then Tukey's Test was used for the estimation of results.

Keywords: Vegetative development, Essential oil, Basil, *Ocimum basilicum*

**P.126. EVALUATION OF TARAMIRA OIL FOR THE SYNTHESIS OF BIODIESEL:
PRETREATMENT AND OPTIMIZATION STUDY USING BIVO4 BASED
PHOTO-CATALYST**

Muhammad Ali¹, Sana Sadaf², Javid Iqbal²

Department of Chemistry, University of Agriculture, Faisalabad-Pakistan

²Punjab Bioenergy Institute (PBI), University of Agriculture, Faisalabad-Pakistan.

Corresponding author Email: m0hammad_al1@aol.com

Use of biodiesel comes with number of advantages over petroleum diesel due to its renewability, superior high biodegradability, low toxicity and negligible sulfur contents. The *Eruca Sativa* (Taramira) oil used for the production of biodiesel. Variable parameters were optimized for the treatment of free fatty acids present in the sample oil. The response to surface methodology (RCM) is employed for the process of optimization of influencing reaction parameters i.e. methanol to oil ratio, catalyst dose, stirring speed and reaction time. About 90% of free fatty acids have been successfully esterified by using 1:1 methanol to oil ratio, 1% catalyst, and 7 hours of contact time with stirring speed of 400 rpm. The efficiency of Nanocomposite synthesized for the pretreatment of sample oil free fatty acids found as 1% > 3% > 5% > 7%. The physico-chemical properties of this Bio-fuel such as specific gravity, density, cetane number and flash point indicate toward a promising source for the generation of renewable bioenergy source.

Keywords: Biodiesel, Free fatty acids, Process optimization

P.127. PREPARATION OF NOVEL SUPPORT FOR CATALYSTS IN BIODIESEL PRODUCTION

Rida Tariq and Muhammad Asif Hanif

Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

The most common way used for biodiesel production is catalytic transesterification process. Usually the recovery of catalyst is difficult from biodiesel but with the use of supports along catalyst make it easier to recover the catalyst from the reaction media after biodiesel production. In the present work, different supports for the catalyst were produced to carry out transesterification process efficiently. Different kinds of supports such as zirconia, zinc oxide, plaster of Paris, silica, and alumina were produced to enhance the efficiency of catalysts as well as the recovery process. Supports were prepared by the complete procedure which involved different steps; precipitation, washing, drying, shaping, impregnation with solution of active component, drying, decomposition, activation and at the end support were produced. Then gas chromatographic-mass spectrometric (GC-MS) analysis was performed to determine the composition of produced biodiesel. The obtained results were tested using ANOVA with post-hocTukey HSD test.

Keywords: zirconia, zinc oxide, plaster of Paris, silica, and alumina

P.128. PRODUCTION OF HIGHLY STABLE FATTY ACID METHYL ESTERS

Shafaq Nisar and Muhammad Asif Hanif

Department of Chemistry, University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

Renewable energy has high demand in the market due to the depletion of conventional energy resources and their harmful effect on the environment. Biodiesel is thought to be the best substituent of the petrodiesel as it is renewable in nature. Biodiesel can be produced either by the catalytic process or by the non-catalytic process. Conventionally biodiesel was produced by catalytic transesterification process which has number of drawbacks such as sensitivity to high free fatty acids as well as to water, complicated processes for the biodiesel separation and purification. In the present study, an innovative and different approach was used for biodiesel production in the absence of catalysts. In this approach desired product is obtained by increasing the temperature of reaction media. Temperature range used to carry out this method for biodiesel production was 543-573 K. Composition of produced biodiesel was analyzed with the help of Gas Chromatographic Mass Spectroscopy (GC-MS) and obtained results were tested statistically by one way analysis of variance followed by post-hoc Tukey test. From the results, it is observed that the highest FAME yield (71.54%) was obtained at temperature of 563 K.

Keywords: Biodiesel, Superheated methanol vapor, GC-MS, Renewable energy

**P.129. DESIGN AND SYNTHESIS OF EUGENOL DERIVATIVES AS
POTENT ANTIOXIDANTS**

Suman Tahir, Muhammad Asif Hanif and Muhammad Irfan Majeed

Department of Chemistry, Faculty of Sciences,

University of Agriculture, Faisalabad-38040-Pakistan

Corresponding author Email: drmuhammadasifhanif@gmail.com

Essential oils are natural, volatile oil of medicinal and aromatic plants. There is increasing concern in extracting and characterizing bioactive natural compounds and using them in various food and pharmaceutical application. Clove essential oil is considered to be one of the most prominent members of the aromatic oils family due to its wonderful scent attributes and greater natural activities. Eugenol is volatile, clear to pale yellow liquid, the major bioactive compound of clove essential oil. Clove were collected from the local market of Faisalabad. The essential oil from clove was isolated using high efficiency distillation process. Then these fractions of clove essential oil were analyzed by Gas Chromatographic Mass Spectrometer (GC-MS). Isolated fractions were chemically derivatized by esterification. The biological activities of clove essential oil and its fraction were determined by antioxidants and antimicrobial parameters.

Keywords: Clove oil, GC-MS, Biological activities, Eugenol, Aromatic plants

**P.130. EFFECT OF LEAD-RESISTANT RHIZOBACTERIA ON GROWTH
AMELIORATION OF *ZEAMAYS* L. IN LEAD CONTAMINATED SOIL**

Zain Fatima and Ambreen Ahmed*

Department of Botany, University of the Punjab, Quaid-e-Azam Campus,
Lahore 54590, Pakistan

Corresponding author Email: ambreenahmed1@hotmail.com

Lead is a toxic non-essential trace element that is discharged via industrial effluents and through paint industries into soil, thereby polluting it. Augmented use of lead nitrate in various industrial processes has contributed towards environmental pollution. There are various chemical means to remediate these soils polluted with lead compounds but use of microbes as bioremediators provides a natural way to reduce toxicity of lead nitrate. Applications of these lead nitrate tolerant PGPRs may decrease the global dependence on chemicals to reduce lead toxicity and provides an ecofriendly, cost effective and green approach to reduce hazardous effects of lead and also improves plant growth in contaminated areas. In the present study eight lead resistant bacterial strains were selected (E31, E32, a1, C1, C2, C3, 3B and 4C) for study. In order to study the impact of these lead-tolerant bacteria, a plant growth experiment was conducted with *Zea mays* with different concentrations of lead stress (500, 1000, 1500 and 2000µg/ml). Various growth, biochemical and anatomical parameters of all treated plants including shoot length, root length, number of leaves, fresh weight, chlorophyll estimation and protein content were compared with non-inoculated control treatments. Such rhizobacterial strains may aid in enhancing the production of crop plants in natural areas that are under lead stress or areas where soil is polluted due to toxic lead compounds.

Keywords: PGPR, lead stress, *Zea mays*

P.131. HYPOGLYCEMIC, ANTIOXIDANT, ANTIHYPERLIPIDEMIC, RENOPROTECTIVE AND HEPATOPROTECTIVE POTENTIAL OF *ALLIUM SATIVUM* L. RHIZOMES INVESTIGATED IN DIABETES RAT MODELS

Sajila Sharif, Fatma Hussain, Muhammad Shahid

Department of Biochemistry, Faculty of Sciences,
University of Agriculture, Faisalabad-38040-Pakistan

Health-promoting perspective of *Allium sativum* L. (Liliaceae), also known as garlic rhizome is attributed to its rich phytochemistry. The current investigation was undertaken to evaluate methanolic extract of rhizome for hypoglycemic (fasting blood glucose, HbA1c), antioxidant (catalase, superoxide dismutase, reduced glutathione lipid peroxidation), antihyperlipidemic (triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol), hepatic (aspartate transaminase, alanine transaminase, alkaline phosphatase, glutamyl transpeptidase, total plasma proteins), and renal (urea, creatinine, albumin, total urinary protein) protective potentials in normal and diabetic groups. Significant decrease in blood glucose, HbA1c, triglycerides, total cholesterol, LDL cholesterol, alanine transaminase, alkaline phosphatase was observed in diabetic rats treated with *Allium sativum* rhizome extracts as compared to untreated sample. Elevated catalase concentrations were measured in diabetic samples after treatment. It is concluded that the *Allium sativum* rhizomes must be considered as excellent candidate for future studies on diabetes mellitus.

Keywords: *Allium sativum*, hypoglycemic, antihyperlipidemic, hepatoprotective

**P.132. BIOGENIC SYNTHESIS, PHOTOCATALYTIC AND ANTIMICROBIAL
ACTIVITY OF MANGANESE NANOPARTICLES**

Urooj Kamran¹, Haq Nawaz Bhatti^{1*}, Munawar Iqbal², Saba Jamil¹ and Muhammad Zahid¹

¹Department of Chemistry, University of Agriculture, Faisalabad, Pakistan

²Department of Chemistry, University of Lahore, Lahore, Pakistan

Corresponding author Email: hnbhatti2005@yahoo.com; haq_nawaz@uaf.edu.pk

Biogenic synthesis of nanoparticle (NP) has attained the wider interest in view of various advantages like simplicity, eco-friendly nature, rapidity and cost-effectiveness. In current investigation, manganese nanoparticles (MnNPs) were fabricated using *Cinnamomum verum* bark extracts (CVBE) using manganese(II) acetate tetrahydrate as Mn precursor. The biosynthesized MnNPs were characterized by SEM, TEM, XRD and FTIR techniques. The synthesized face centered cubic MnNPs were less than 100 nm in size, spherical, crystalline and in aggregate form. The photocatalytic activity (PCA) of MnNPs was evaluated by degrading Congo Red (CR) dye. At optimized condition of pH 7.0, 0.06 g/L MnNPs dose, 10 mg/L initial dye concentration and UV irradiation time of 60 min, the CR dye degradation was 78.5%, which indicates the photocatalytic potential of biosynthesized MnNPs. The antimicrobial activity of biosynthesized MnNPs was tested against *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) strains. Results revealed that the potential applications of the indigenous medicinal plants for the fabrication of NPs and their subsequent application in the field of nanotechnology, which could possibly be extended for the synthesis of other metals NPs. The biosynthesized MnNPs would contain natural anti-microbial agents, which may serve for the production of potential antimicrobial agents.

Keywords: Biosynthesis; *Cinnamomum verum* bark; Mn nanoparticles; Congo Red dye; Photocatalytic activity; Antimicrobial activity

**P.133. ANTIOXIDANT ANALYSIS OF ROOT BIOACTIVES OF CANOLA PLANT
EXUDED IN RESPONSE TO ABIOTIC STRESS**

Saba Ghufuran, Bushra Sultana*, Sadaf Yaqoob, Tehmina Sharif, Zunaira Jabeen

Department of Chemistry, University of Agriculture, 38040 Faisalabad, Pakistan.

Plants have an astonishing function of formulating and releasing organic and inorganic compounds into the rhizosphere in response to various biotic and abiotic stresses. These compounds not only impart specific functions to plants but also act as natural sources of strong antioxidants. Canola (*Brassicaceae*) is useful in the treatment of various diseases like cancer, diabetes, arthritis, many inflammatory and cardiovascular diseases etc. This plant has a remarkable applicability in pharmaceutical, food and cosmetic industries. The purpose of current research was to analyze the antioxidant activity of root exudates of canola plant under the influence of abiotic stress such as physical (UV radiation with 2 different wavelengths 254 nm and 364 nm) and chemical stress (Tween 20 at 3 different concentrations 2, 4 and 6%). Total phenolic content, DPPH free radical scavenging activity and reducing power of root exudates of canola were determined. Two-way ANOVA was employed in order to analyze the results. According to the results, canola root exudates showed the range for TPC 12.68 ± 0.32 - 576.63 ± 14.42 mg GAE/g of DW. The results of DPPH assay indicated that the IC_{50} values ranged 0.51 ± 0.02 - 0.72 ± 0.03 μ g/mL for the root exudates of canola. While, the reducing power of root exudates of canola plants was determined to be in a range 0.32 ± 0.01 - 0.88 ± 0.02 mg/100 mL. It was concluded that UV radiation of 254 nm wavelength with 180 minutes treatment time and 2 days collection time showed the highest antioxidant activity.

Keyword: Canola, Root exudates, Antioxidant activity, Total phenolic contents

**P.134. VIRTUAL ANALYSIS OF HEAT STRESS TRANSCRIPTION
FACTOR IN PLANTS BY SNPS IDENTIFICATION**

Sumaira Rasul^{1*}, Farha Anwer¹, Kashif Aslam¹, Fozia Saeed¹, Rehana Iqbal², Muhammad Shahzad Anjum¹ and Hamid Manzoor¹

¹Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University,
60800
Multan, Pakistan.

²Institute of Pure and Applied Biology, Bahauddin Zakariya University, 60800
Multan, Pakistan.

Corresponding author Email: dr.sumaira@bzu.edu.pk

Homology is the basic concept of shared evolutionary ancestry and describes the relation between different individuals placed in different genera in the history of evolutionary biology. It is the fundamental concept of comparative biology that describes the ideas of genomics and developmental biology. In current study, homology analysis of of total 175 gene sequences was done through BLASTN. Multiple sequence alignment and identification of SNPs of 175 sequences were done by using UGENE software. Phylogenetic analysis of nucleotides of total 118 selected sequences was done by MEGA5. Entirely, 175 sequences containing *HSFA2* gene was downloaded and BLASTN homology analysis gave $\geq 90\%$ similarity index. Multiple sequence alignment and SNPs identification was done via unipro UGENE. A total of 118/175 sequences were selected having 28 reference sequences for the identification of deleterious SNPs from SIFT web server. Two species of *Musa acuminata* showed best deleterious SNPs that affect protein function. Interactive proteins were searched from STRING online server and interactive proteins selection was based on negative E-value then results preceded towards docking. Protein docking was done through HDOCK server by using reference and mutated sequences with interactive protein and results showed RMSD score. The RMSD values from our results were 196Å and 152 Å, 186 Å and 287 Å. The results showed the variation in structural configuration of protein with mutated protein that also has affect the function of a protein.

Keywords: Heat Stress Transcription Factor, Multiple Sequence Alignment, Single Nucleotide Polymorphism, Deleterious SNPs, Protein-Protein Docking

P.135. POTENTIAL OF DIFFERENT GENERATIONS OF CEPHALOSPORINS TO INHIBIT BIOFILM OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

Jawaria Aslam, Muhammad Shahid and Hina Fatima

Department of Biochemistry, University of Agriculture, Faisalabad

Corresponding author Email: hinafatimauaf@yahoo.com

Microbial Biofilms are structured communities of bacteria which are attached to a surface and embedded or adhered in a self-produced matrix of extracellular polymeric substances (EPS). Microbial biofilms exhibit increased tolerance towards antibiotics and are an important dilemma in clinical settings in which they are main cause of chronic infections. Novel therapeutic strategies are needed to develop anti-biofilm efficacy and support reduction in antibiotic use. In present research project, we studied the efficacy of different generations of cephalosporins to disrupt the biofilm of two pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. The maximum percentage of biofilm disruption against *S. aureus* by 1st, 2nd, 3rd and 4th generations of cephalosporin was 71.63%, 78.8%, 42.56% and 60.63%, while against *E. coli* it was 46.27%, 52.83%, 64.39% and 66.47% respectively. Furthermore, our findings showed that cephalosporins displayed a significant disruption on the established biofilm produced by *S. aureus*, but not by *E. coli*. Characterization of disrupted biofilm was also carried out through Fourier transformed infrared (FTIR) spectroscopy and scanning electron microscopy (SEM).

Keywords: Biofilm, cephalosporins, *Staphylococcus aureus* and *Escherichia coli*

P.136. A COMPREHENSIVE STUDY ON GROWTH SUSTAINABILITY OF MICROALGAE AND UNLOCKING OF ITS MULTILAYERED ADVANTAGES

Haq Nawaz Bhatti¹, Munazza Maqbool¹ and Sana Sadaf²,

¹Department of Chemistry, University of Agriculture, Faisalabad-Pakistan

²Punjab Bioenergy Institute (PBI), University of Agriculture, Faisalabad-Pakistan

Corresponding author Email: hnbhatti2005@yahoo.com; munazzachemist@gmail.com

The current study was designed to explore the multifold advantages of microalgae by its cultivation in municipal wastewater for biomass production, municipal wastewater treatment, bioenergy production and use of residual biomass for wastewater treatment. The study results revealed that *Chlorella* strain has the ability to reduce 85.5 % nitrogen, 88 % phosphorus and 86.4 % chemical oxygen demand from municipal wastewater. The microalgae biomass was used in biodiesel production and residual biomass was used for adsorptive removal of congo red dye. It was observed that microalgae residual biomass has best dye removal capacity in polypyrrole composite form (772.7 mg/g). Kinetic study results revealed the best fitness of pseudo second order kinetic model. Thermodynamic study results revealed that biosorption of test dye onto microalgae biomass is a feasible process and is exothermic in nature. The biomass was characterized by FT-IR, SEM and pH_{PZC} determination. The study results revealed that *Chlorella* has great potential to grow in municipal wastewater by taking up nutrients present in it. This not only helps in sustainable cultivation of microalgae but it also helps in avoiding eutrophication. Moreover, after bioenergy applications, the waste biomass can also be used as an efficient material for treatment of textile effluents.

Keywords: *Chlorella*; Municipal wastewater; Congo Red dye; Biosorption; Kinetic study

**P.137. OPTIMIZATION OF QUERCETIN LOADED PLGA NANOPARTICLES,
THEIR ANTIBACTERIAL POTENTIAL AND COMPARATIVE
PHARMACOKINETICS**

Aroosa Fazalillahi, Faqir Muhammad* and Bushra Akhtar

Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad,
Pakistan

Corresponding author Email: Aroosafazalillahi1403@gmail.com

Drug resistance against various microorganisms is an alarming problem now a days. Quercetin, bioactive flavonoid, have been reported to possess antibacterial potential. But the therapeutic applications of quercetin are restricted due to poor solubility & bioavailability. The incorporation of quercetin in biodegradable polymer, poly lactic co-glycolic acid (PLGA) nanoparticles resolved the issues. Quercetin PLGA NPs prepared by solvent displacement method and characterized. These nanoparticles were studied *in vitro* against *E. coli*, and *S. aureus* using broth dilution method. Prepared NPs exhibited size of 243.3nm, -1.83mV zeta potential and 0.138 polydispersity index. The formulation showed best *in vitro* characteristics was subjected to pharmacokinetic (PK) analysis. The comparative PK study of quercetin, pure quercetin dispersion in distilled water and in the form of quercetin loaded PLGA NPs was done on rabbits. Blood sampling was done at different time intervals. The blood samples were analyzed using UV-visible spectrophotometric analysis. PK parameters were evaluated by using PK solver. A comparison was done b/w PK parameters of pure and of quercetin loaded PLGA NPs. The values of area under curve, clearance, volume of distribution, time to peak concentration (T_{max}), peak concentration (C_{max}) were 149.14±23.94hµg/mL, 0.155±0.01 (mg)/(µg/ml)/h, 3.795±0.78(mg)/(µg/ml), 9.8±0.28h, and 7.8±1.24µg/mL respectively for pure quercetin and were 898.17±45.51hµg/mL, 0.03±0.002(mg)/(µg/ml)/h, 0.49±0.03(mg)/(µg/ml), 13.7±0.34h, and 28.42±1.21µg/mL. The above study thus clearly indicates better absorption and enhanced PK parameters of quercetin loaded PLGA NPs.

Keywords: Quercetin, PLGA, Nanoparticles, Pharmacokinetic parameters

P.138. BIOACTIVE AND THERAPEUTIC POTENTIAL OF *SMILAX CHINA* L. ROOTS

Fatma Hussain and Sunder Unab

Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: fatmauaf@yahoo.com

Smilax china (China root) indigenous to China and Japan has been used in Chinese traditional medicine. This study was designed to explore selected pharmacological potential of *S. china* root extracts prepared in six different solvents (methanol, *n*-butanol, *n*-hexane, chloroform, ethanol and ethyl acetate). *In vitro* study involved the assessment of antioxidant potential by total phenolic content (TPC), total flavonoid content (TFC) and 2, 2-diphenyl 1-picrylhydrazyl (DPPH) radical trapping potential. Antidiabetic (antiglycation, acetylcholinesterase, alpha glucosidase and alpha amylase inhibitions), antibiofilm, mutagenicity (ames and brine shrimp assay) cytotoxicity (hemolytic and DNA damage protection assays) along with thrombolytic and antimicrobial (antibacterial and antifungal) activities were performed. All data was analyzed by ANOVA and inference after ANOVA. TFC and TPC values were 1.09 to 22.6 mg CE/100 g and 6.86 to 24.06 mg GAE/100 g respectively. DPPH scavenging potential was in range 3.7 to 34%. Ethanol exhibited significant glycation (70%) whereas ethanol (7.49%), *n*-hexane (90.15%) and methanol (29.7%) indicated maximum acetylcholinesterase, alpha glucosidase and alpha amylase inhibitions respectively. Prominent microbial growth restrain potential (58%) was shown by *n*-butanol fraction against *Pasturella multocida*. Ethanol and ethyl acetate fractions were potent antimutagenic against TA98 while methanol and ethanol showed antimutagenicity against TA100. In brine shrimp lethality *n*-butanol fraction showed most active larvicidal effect. *n*-hexane fraction showed optimum hemolytic potency (20%) whereas, DNA damage was suppressed by ethanol, ethyl acetate, *n*-hexane and *n*-butanol fractions. Highest thrombolytic activity (32%) was exhibited by aqueous fraction. Prominent (15 mm) zone of inhibition against *Staphylococcus aureus* was exhibited by ethyl acetate fraction while optimum (8 mm) zone of inhibition against *F. solani* were shown by chloroform fraction. *In vivo* assessment revealed the noteworthy antidiabetic, antioxidant, hypolipidemic, hepatoprotective and renoprotective potential of *S. china* root extracts in diabetic animal models. It is suggested that this plant is considerable to be used as pharmaceutical dietary supplements as well as chemo preventive agent.

**P.139. BIOACTIVE AND THERAPEUTIC POTENTIAL OF
GLYCYRRHIZA GLABRA L. ROOTS**

Fatma Hussain and Irum Iqbal

Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: fatmauaf@yahoo.com

Glycyrrhiza glabra L. known as liquorice is herbal plant having worldwide applications for its usage in food industry, pharmaceutical products and alcoholic beverages. In this study, for methanol and ethanol fractions of roots of *G. glabra* DPPH (2, 2-diphenyl 1-picrylhydrazyl) scavenging assay, total phenolic content (TPC), total flavonoid content (TFC), enzymes inhibition (acetyl cholinesterase, alpha amylase, alpha glucosidase), glycation inhibition, cytotoxic, thrombolytic, microbial, mutagenic and biofilm inhibition assays were performed. Results showed that DPPH radical scavenging ability was in range of 5.08-46%. Whereas, 80.96 mg GAE/100 g TPC and 38.96 mg CE/100 g TFC, 85.90% glycation, alpha amylase, acetyl cholinesterase and alpha glucosidase inhibitions respectively for antidiabetic evaluation. Methanol (35.4%) and ethyl acetate (19.46%) extract being the most potent thrombolytic and hemolytic agents respectively whereas DNA damage protection was shown by methanol, ethanol, ethyl acetate and chloroform extracts. Ethanol and ethyl acetate fractions revealed non-mutagenic efficacy by Ames test whereas maximum cytotoxic potential through brine shrimp activity was shown by chloroform (90%) extract. All extracts revealed significant potential against bacterial and fungal growth. Similarly ethyl acetate exhibit noteworthy percentage imperviousness against biofilm formation of *Pasteurella multocida* (72%) and ethanol (51.57%) against *Staphylococcus aureus*. *In vivo* study evaluation on diabetic animal's models perceived significant antidiabetic, antioxidant, antihyperlipidemic and reno-hepatic profile. In conclusion, *G. glabra* root extracts have remarkable activity to fight against oxidative stress, hyperglycemia, blood coagulation, bacterial growth, DNA damage and also showed exceptionally useful therapeutic results through *in vivo* study against diabetic animal models. Further studies need to be conducted to evaluate its more biochemical parameters.

**P.140. A STUDY OF LEVEL OF VITAMIN D, CALCIUM, PHOSPHOROUS AND
PARATHYROID HORMONE IN SCHOOL CHILDREN**

Nazia Akhtar, Muhammad Faizan and Tahira Iqbal

Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: najiasial@gmail.com

Vitamin D is the prohormone that play a significant role in maintaining the bone health and the level of metabolic bone markers in blood circulation. It can be provided via dietary intake and formed in skin by exposure to sunlight. Deficiency of vitamin D is increasing on each successive day in the population of developing countries, but it appears much worse in Pakistan particularly among school going children in spite of sufficient sunlight. Main objective was to estimate the vitamin D level in relation with bone markers such as Ca, P and Parathyroid Hormone among the school going children. A study was conducted among school children of age 10 to 15 years old from different classes of the schools of Faisalabad. Blood samples of children was collected and level of vitamin D in these blood samples was measured by using High performance liquid chromatography (HPLC). The serum level of Ca and P was measured through spectrophotometry. While, the PTH analysis was done via ELISA technique. For statistical analysis, analysis of variance (ANOVA) was applied to analyze the results of experiments. The Mean $\pm$ SD of the results of vitamin D is 13.06, Calcium level is 0.55, Phosphorous is 1.079 and PTH is 3.028 in the blood serum of children. This showed that how the values of these 4 bone markers vary in accordance with one another. So, there is need of awareness of vitamin D intake and its fortification of food to overcome the deficiency in kids.

P.141. TECHNETIUM-99M LABELED IBUPROFEN: DEVELOPMENT AND BIOLOGICAL EVALUATION USING STERILE INFLAMMATION INDUCED MICE MODEL

Naeem-Ul-Haq Khan¹, Syed Ali Raza Naqvi¹, Samina Roohi²

¹Department of Chemistry, Government College University, Faisalabad-38000, Pakistan

²Isotope Production Division (IPD), Pakistan Institute of Nuclear Science and Technology (PINSTECH), Nilore, Islamabad-Pakistan

Corresponding author Email: naeemulhaqk@student.unimelb.edu.au

In this study we are presenting the development of technetium-99m (^{99m}Tc) labeled ibuprofen for the imaging of aseptic inflammation. ^{99m}Tc-Ibuprofen complex was developed by optimizing the radiolabeling conditions such as reaction time, ligand and reducing agent concentration, pH, reaction time and temperature. After addition of 600 µg of ibuprofen, 4 µg of stannous chloride as reducing agent and about 300 MBq ^{99m}Tc radioactivity, the mixture was allowed to react for 15 min at room temperature and 11 pH. Chromatography analysis revealed >94% ^{99m}Tc ibuprofen complex formation with promising stability in saline and blood serum up to 6 h. Biodistribution study using normal and sterile inflammation induced mice indicated low accumulation of labeled compound in major body organ, however, kidneys (14.76 ± 0.87 % ID/g organ) and bladder (31.6 ± 3.0 % ID/g organ) showed comparatively higher radioactivity due to principal excretory path. Inflammation tissue to normal tissue ratio (T/NT) at 1 h showed significant value (4.57 ± 0.56). Radiochemical purity of ^{99m}Tc-ibuprofen and biological evaluation using sterile inflammation induced mice revealed the agent bears promising potential to locate the aseptic inflammation which can be utilized to discriminate the septic and aseptic inflammation in combination with true septic inflammation agent.

**P.142. REAL-TIME EXPRESSION AND VALIDATION OF MICRORNAS FOR
NON-INVASIVE DIAGNOSIS OF HCV RELATED LIVER CANCER**

Awais Altaf^{1*}, Freeha Fiaz², Azra Quraishi², Iqra Khalid², Ashir Ibrahim Dutt² and Amer
Jamil²

¹Government College University, Faisalabad-Pakistan

²University of Agriculture, Faisalabad-Pakistan

Corresponding author Email: awaisaltaf362@yahoo.com

Hepatic cancer is the third leading cause of death among cancer related mortalities worldwide. HCV (hepatitis C virus) is considered as a major risk factor for the progression of Hepatocellular carcinoma (HCC). An important fact for the development of HCC is the late or poor diagnosis of HCV. MicroRNAs have been exploited to use for the accurate, early, sensitive and specific diagnosis of HCC. Dysregulation of oncogenic miRNAs has been confirmed, not only in diseased tissues but also in plasma, serum and other body fluids as circulating diagnostic markers. This research was conducted to validate the oncogenic microRNAs potential for monitoring and predicting disease progression. Human liver cancer specific microRNAs 122 and 222 were evaluated with a robust, sensitive and a very specific technique for the real-time detection and quantification of their expression level in chronic hepatitis C (CHC) related HCC patients and controls. The comparison of Ct values ($2^{-\Delta\Delta Ct}$) of diseased and healthy persons figured out the relative quantification of expression levels of miRNA. A significant elevation in levels of both miR-122 and miR-222 was observed in chronic HCV patients leading to carcinoma. Along with many clinicopathological factors of chronic hepatitis C, a substantial correlation ($P < 0.05$) was found among both microRNAs. The mortality rate is high because of late or poor diagnosis of chronic hepatitis which turns into liver cancer afterwards. This death rate may be decreased by commercializing the microRNA based detection kits, after further experimentation and verification.

Keywords: microRNAs, diagnostic biomarker, hepatocellular carcinoma.

P.143. IRON OXIDE NANOPARTICLES BASED SURFACE-ENHANCED RAMAN SPECTROSCOPIC (SERS) PROTOCOL FOR DIAGNOSIS OF HEPATITIS C

Muhammad Irfan Majeed*, Haq Nawaz, Muhammad Kashif, Saifullah and Saqib Ali

Department of Chemistry, University of Agriculture Faisalabad, Pakistan.

Corresponding author Email: irfanmajeed2003@gmail.com

Surface-enhanced Raman spectroscopy (SERS) has acquired great attention as an advanced spectroscopic analytical technique due to its rapid, non-destructive and ultratrace analyte-detection capabilities. While nanomaterials due their small size, high surface area and unique optical and electronic properties have also been the focus of research in disease diagnosis and treatment in recent decades. It has been well established that nanoparticles such gold, silver, iron oxide etc. can cause the great enhancement in Raman signals of an analyte. Early diagnosis is considered as very critical for disease management and treatment in case of dangerous viral diseases such as hepatitis C and patient's blood plasma is considered as a good medium for detection of virus itself or disease related biomarkers leading to its early diagnosis. Here in we report the development of iron oxide modified quartz substrates for the early and rapid detection of hepatitis C from the patient's blood plasma. Initial results of our developed SERS protocol with iron oxide nanoparticles have shown great enhancement in Raman signals in comparison to the simple Raman analysis of patient's blood plasma for hepatitis C virus. Furthermore, it is assumed that in future this method may provide a better alternative for early and rapid diagnosis of hepatitis C infection due to its higher sensitivity, ease, speed and low cost in comparison to the conventional ELISA and PCR techniques.

Keywords: Nanoparticles, iron oxide, hepatitis C, Raman, surface-enhanced, SERS.

P.144. MULTIVARIATE DATA ANALYSIS OF RAMAN SPECTRAL DATA OF HEPATITIS C INFECTED BLOOD PLASMA SAMPLES.

Haq Nawaz ^{a*}, Muhammad Irfan Majeed ^a, Saqib Ali ^a, Saifullah^a and Muhammad Kashif ^a

^a Department of Chemistry, University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: haqchemist@yahoo.com

In the current study, Raman spectroscopy (RS) is employed for the characterisation of the HCV positive blood plasma samples. RS is found helpful for the identification of the biochemical changes taking place during the development of Hepatitis C. Principal Components Analysis (PCA) of the Raman spectral data leads to the identification of the Raman spectral markers of the Hepatitis C Virus (HCV) infection. Raman spectral changes including 720 cm⁻¹, 1077 cm⁻¹, 1678 (C=O stretching mode of dGTP of RNA), 1778 cm⁻¹ (RNA), 1641 cm⁻¹(amide-I/proteins), 1721 cm⁻¹(C=C stretching of proteins) and lipids at 1738 cm⁻¹(C=O of ester group in lipids) are observed solely in the HCV positive samples. These Raman spectral peaks of blood plasma samples of the patients can be associated with the development of the biochemical changes during HCV infection.

**P.145. DETOXIFICATION OF AFLATOXIN B₁ BY *LENTINULA EDODES*
(SHITAKE) IN BROILER CHICKEN**

Zinayyera Subhani¹, Muhammad Shahid¹, Muhammad Naveed², Sadia Aslam³, Asma
Irshad⁴, Muhammad Salman Sarwer¹, Shuja-ur-Rehman¹

¹ Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

² Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad,
Pakistan

³Department of Biochemistry, Government College Women University, Faisalabad, Pakistan

⁴Center of Excellence in Molecular Biology, University of Punjab, Lahore, Pakistan

Corresponding author Email: zainisub@gmail.com

Aflatoxin B₁ induced renal impairment is not adequately studied regarding its extent and preclusion. The aim of this study is to estimate the effect of AFB₁ on renal tissue and whether its harmful effect could be prevented by the nature blessed, conventional antioxidant *Lentinula edodes*. Hundred and twenty 7-d old broiler chicks (Hubbard) were randomly divided into six groups with two replicates having 10 birds each. Group 1 served as control, Group 2 intoxicated with aflatoxin B₁. Groups 3 and 4 administered *Lentinula edodes* (LE; 5% and 10% per kg feed respectively), while Groups 5 and 6 administered with aflatoxin B₁ (350 ppb/kg feed) along with both LE (5% and 10% per kg feed). The study was conducted for 35 days. Protective efficacy was determined by evaluating the levels of serum urea, creatinine and also by lipid peroxidation (LPO), total antioxidant status (TAS), non- enzymatic antioxidant reduced glutathione (GSH) and activities of enzymatic antioxidants glutathione reductase (GR), catalase (CAT), glutathione- S-transferase (GST), glutathione peroxidase (GPX) in kidney. Significant reduction in the activities of antioxidant enzymes and non-enzymatic antioxidant system in AFB₁ treated chicks as compared to control could be responsible for increased LPO levels during AFB₁ induced kidney damage. The results showed increased urea and creatinine levels as well as reduction sodium concentrations in plasma of AFB₁ treated chicks. Nephro-protective effects observed in percentage dependent manner. These data elucidate the protective efficacy of *Lentinula edodes* against pro-oxidant AFB₁ induced nephrotoxicity might be due to its antioxidant and free radical scavenging activity.

Keywords: *Lentinula edodes*; aflatoxin B₁, broiler, antioxidants, nephrotoxicity

P.146. DETERMINATION OF METRONIDAZOLE INDUCE OXIDATIVE DAMAGE IN LIVER, KIDNEY AND GILLS OF *OREOCHROMIS NILOTICUS*

Asif Saleem¹ and Kashif Jilani²

¹Department of Zoology and Fisheries, ²Department of Biochemistry,

University of Agriculture, Faisalabad

Corresponding author Email: kashif.jillani@uaf.edu.pk

Metronidazole belongs to nitromidazole class of antibiotic, which is used to treat bacterial diseases related to skin and intra-abdominal systems. Basic regulatory processes of the organisms are damaged by the unbalancing activities between the oxidants and anti-oxidants. Oxidation in the body may leads to the damaging of different organs. To deal with the oxidative stress there are anti-oxidant enzymes system present in the cells which is mainly comprises of glutathione peroxidase, Superoxide dismutase and Catalase. *Oreochromis niloticus* is one of the most important species of fish of Pakistan due to the economical point of view. In the present study oxidative damaged was investigated in kidney, liver and gills of *Oreochromis niloticus*. One year old fingerling of *Oreochromis niloticus* was used to treat with the concentrations i.e (150 mg/L and 300 mg/L respectively) of metronidazole for the investigation of oxidative stress. Liver, kidney and gills of fish was dissected after the 15 days exposure to metronidazole. Antioxidant enzymes activities were measured in isolated organs of fish and compared with the activities in the organs of the control group. During the experiment different physico-chemical parameters i.e. temperature, pH and total hardness were measured on regular basis. It is observed that the anti oxidants enzymes activities were found significantly less in exposed organs of the fish which is the confirmation of oxidation due to metronidazole treatment. One way ANOVA and Tukey's test as post tests, were used for the statistical analysis.

Keywords: Metronidazole, *Oreochromis niloticus*, oxidation

P.147. THE PHYSIOCHEMICAL COMPOSITION OF DATE PALM FRUITS AT THREE DIFFERENT EDIBLE STAGES

Muhammad Waseem^{*a}, Summar Abbas Naqvi^a, Muhammad Jafar Jaskani^a, Muhammad Shahid^b Hina Fatima^b, Iqar Ahmad Khan^a

^aPomology Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad 38040, Pakistan; ^bProtein Molecular Biology Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: wasimm45@gmail.com

The fruit of date palm (*Phoenix dactylifera* L.) has rich source of nutrition for humans as it provides a large amount of sugars, minerals, fibers, vitamins, phytonutrients, fatty acids, protein and amino acids. The objective of the present study was to investigate the effect of maturity on the physiochemical composition of date palm fruits. Fifteen varieties were taken from KPK and their physicochemical analysis were done at three different maturity stages (khalal, rutab and tamar). The analysis fruits revealed that the antioxidant activity (65-90 %), total phenolic contents (420.28 to 1502.73 mg GAE/100g, FW), total flavonoids (19.67 to 77.12 mg CE/100g, FW), ascorbic acid (21.80 to 57.41 mg/100, FW), total anthocyanins (0.10 to 0.52 mg/100g, FW), total carotenoids (62.3 to 91.25 mg β -carotene equivalent/100g, FW), antioxidant enzymes (SOD, POD and CAT) and the soluble protein contents (5.73 to 2.75 g/100g) were higher in higher at khalal stage but decreased at fully ripened (tamar) stage. Moreover, total sugar contents (35.47 to 48.17 mg/100g, FW), reducing sugars (19.98 to 32.85 mg/100g, FW) and minerals (nitrogen, phosphorus and potash) were lower at khalal stage and increased from rutab to tamar stage. It was found that the non-reducing sugars were present only at khalal and rutab stage. Gulistan, Haq Nawaz, Basra and Dhaki cultivars revealed best overall values in examined compounds. Our results revealed that variation in different biochemical attributes is mainly depended on the difference in fruit maturity stage and cultivar. Date palm cultivation can be expended by selecting cultivars exhibiting high values of beneficial biochemical attributes.

**P.148. EVALUATION OF GROWTH PROMOTING POTENTIAL OF PLANT
GROWTH PROMOTING PSYCHROPHILES FROM GILGIT-BALTISTAN**

Saboor-ul-Hassan and Ambreen Ahmed*

Department of Botany, University of the Punjab, Quaid-e-Azam Campus,
Lahore 54590, Pakistan

Corresponding author Email: ambreenahmed1@hotmail.com

The global challenges of food demand in today's world require crop improvement which needs improved soil fertility. The use of synthetic fertilizers to make the soil fertile and nutrient rich have raised many environmental issues. In addition to this, they are not retained in slopy surfaces in hilly areas. Application of plant growth promoting bacteria is a healthy and environment friendly approach in this regard to replace chemical fertilizers. Most of the PGPRs isolated from the areas with climatic condition involving high temperatures are unable to survive in colder region so the current study deals with the isolation of psychrophilic plant growth promoting bacteria from Gilgit-Baltistan. Three rhizospheric psychrophiles isolated from Gilgit- Baltistan were characterized morphologically and physiologically. The strains were analysed for their temperature range. The growth cycle of the isolated strains was also studied. These three psychrophilic and three mesophilic isolates were used to treat seeds of *Triticum aestivum* and growth parameters of treated and non-treated plants were analysed. Psychrophiles proved to be good biofertilizers which can be used in cold and hilly areas where other chemical fertilizers can't be retained in soil for longer time as well as to preserve the environment from damages caused by chemical fertilizers.

Keywords: *Triticum aestivum*, rhizosphere, biofertilizer, mesophiles, pshycrophiles

P.149. ROLE OF ENDOCRINE DISRUPTING CHEMICALS IN THE METABOLISM OF CARBOHYDRATES: INSERTING THE ASSOCIATION INTO PERSPECTIVES

Muhammad Sajid Hamid Akash¹, Shakila Sabir^{1,2}, Fareeha Fiayyaz^{1,3}, Kanwal Rehman⁴

¹Department of Pharmaceutical Chemistry, ²Department of Pharmacology,

³Department of Microbiology, Government College University Faisalabad, Pakistan.

⁴Department of Pharmacy, University of Agriculture, Faisalabad, Pakistan.

Corresponding author Email: sajidakash@gcuf.edu.pk, sajidakash@gmail.com

Endocrine disrupting chemicals (EDCs) are widely spread globally. From the last two decades, EDCs contamination has been increased dramatically due to continuous massive discharge in the sewage and industrial effluents. EDCs have got researchers' attention as they are strongly associated with a number of endocrine related disorder; particularly diabetes mellitus. Arsenic and cadmium, two most deleterious environmental pollutants, are widely distributed in the environment. Additionally, they induce a number of health hazards. They are stored particularly in the kidney, liver, and pancreas and produce deleterious effects on glycolysis, glucogenesis and gluconeogenesis by disturbing their relevant key enzymes. In addition to decreased insulin production and resistance, impaired hepatic glucose homeostasis also have an important role in diabetes mellitus pathogenesis. EDCs bring the conformational changes in certain glucose metabolizing key enzymes leading to their inactivation. Contrary, EDCs also have potential to up-regulate the expression of few enzymes resulting in their enhanced activity. Additionally, arsenic and cadmium also disturb hormonal balance; directly linked with glucose homeostasis e.g. insulin, glucocorticoids and catecholamines due to disruption of pancreas and adrenal gland. EDCs increase reactive oxygen species level and decrease the anti-oxidative defense mechanism with subsequent disruption of multiple organs including glands. We are briefly focusing on effect of EDCs on carbohydrate metabolizing enzymes and hormones.

Keywords: EDCs, arsenic, cadmium, glycolysis, glucogenesis, gluconeogenesis

**P.150. PRODUCTION OF MICROBIAL POLYHYDROXYALKONATE AS
EFFECTIVE DRUG CARRIER**

Rida Naz¹, Mahwish Salman¹, Muhammad Anjum Zia²

¹Department of Biochemistry, Government College University Faisalabad, Pakistan

²Department of Biochemistry, University of Agriculture Faisalabad, Pakistan

Corresponding author Email: ridanaz5969@gmail.com

Polyhydroxyalkonate (PHA) is a biodegradable and biocompatible microbial polymer. During the conditions of limiting nutrients and in the presence of large carbon source bacteria make PHAs. Energy storage compounds are broken-down and consumed to fulfill energy requirements when cell has limited amount of nutrients. PHA use in a drug delivery system, for controlled and targeted drug transportation including, vaccines and for many cancer drugs. Different PHA formulation such as, microsphere, rods, nanoparticles, microparticle and functionalize PHA are used in drug delivery. Some other applications of PHA in other life sector like packaging, food industry, agriculture field and as biofuel, making PHA as attractive polymer. Recently PHA is produce by using different low cost carbon sources. Continuously researchers are working to produce a large amount of PHA from low-cost renewable resources due to its broad medical applications.

Keywords: Polyhydroxyalkonate, Drug delivery, Microsphere, Nanoparticles, PHA Rods

P.151. INDUSTRIAL APPLICATIONS OF BIOFILMS

Roman Anwar¹ and Muhammad Shahid^{1*}

Department of Biochemistry, University of Agriculture Faisalabad

Corresponding author Email: mshahiduaf@yahoo.com

Biofilms are an integral part of the natural environment and can also serve as very beneficial purposes in domestic and industry level, such as in the treatment of drinking water, wastewater and detoxification of hazardous waste. Biofilms are beneficial for the production of numerous industrial chemicals such as ethanol, butanol, lactic acid, acetic acid/vinegar, succinic acid, and fumaric acid. It also emphasizes application of biofilm reactors for treatment of dairy industry wastewater, oily sea water, and wastewater in general, Agriculture Industry, Medical & Pharmaceutical Industry. The bioreactor designs where these biofilms are used can be batch, continuous stirred tank (CSTR), packed bed (PBR), fluidized bed (FBR), airlift (ALR), or any other suitable reactor configuration. Biofilm-bioremediation has emerged as a technology of choice for remediating groundwater and soil at many sites contaminated with hazardous wastes. To understand biofilm, numerous direct and indirect experimental approaches have been used to study the biofilms. Molecular genetics approaches have led to study on the process of biofilm development in order to understand its genetic regulations.

P.152. FREQUENCY OF CHRONIC COMPLICATIONS IN DIABETES TYPE 2, IN RURAL AREAS OF SOUTHERN PUNJAB, PAKISTAN

Maryam Pakiza¹, Muhammad Noman Ishaq², Aftab Ahmad³, Zeeshan Nadeem²
and Muhammad Hamza Tariq⁴

¹University of Agriculture Faisalabad, Faisalabad, Pakistan

²Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore 54590, Pakistan

³Centre of Advanced Studied in Agriculture and Food Security, University of Agriculture
Faisalabad, Faisalabad, Pakistan

⁴Atta ur Rehman School of Applied Biosciences, NUST, Islamabad, Pakistan

Corresponding author Email: Maryampakiza07@gmail.com

About half of the diabetic population in Pakistan is unidentified and almost half of the initially characterized diabetic patients are seen to have diabetic complications. The purpose of the current study was to evaluate the frequency of chronic complications of Diabetes Mellitus Type 2 in patients attending a primary care center at Jampur, Pakistan. Place and Time of Study: Patients having their initial appointment at outpatient department of Iqbal Diabetes Clinic, Jampur from February to June 2018. A total of 100 diabetic patients were taken from February to June 2018. A detailed review of medical history, physical inspection and clinical and laboratory tests of blood samples were performed to recognize the dyslipidemia and related complications. A neuropathy disability score greater than zero was used to classify peripheral neuropathy as low/ medium/ high. An ophthalmoscopic detailed observation of dilated retina was performed. All the data was explored statistically with significance at p. Hyperglycemia was observed in 85%, hypertension in 62%, dyslipidemia in 58%, obesity in 52%, retinopathy in 15%, neuropathy in 47%, nephropathy was in 42% of diabetic patients. Obesity, low HDL, High LDL and total cholesterol were significantly higher in females (p at 0). Neuropathy was seen higher in patients with shorter diabetes duration while all other complications were high in patients with longer diabetes duration and of longer age group. The current study will be fruitful for the management and control of secondary complications in diabetic patients of Pakistan.

Keywords: Frequency, Diabetes type 2, chronic complications, neuropathy, retinopathy, nephropathy.

P.153. A STUDY OF LEVEL OF VITAMIN D AND OTHER BONE MARKERS IN FEMALE DIABETIC PATIENTS

Iqra Ishfaq, Nazia Akhtar and Tahira Iqbal

Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

Corresponding author Email: iqra.queshi07@yahoo.com

Vitamin D, the sunshine vitamin, is now well thought-out as a standard, not only for its worth in bone health in children and adults, but also for its health benefits such as dropping the threat of chronic diseases which includes autoimmune disease, osteoporotic disorders, cardiovascular disease and common cancer. This study was carried out to investigate the association between the onset of diabetes in females and the level of vitamin D. In this pursuit, concentrations of Ca^{+2} , P, PTH and the level of vitamin D in the circulating blood have been examined in diabetic female patients and results have supported the hypothesis that there is some association between type 2 diabetes and circulating concentrations of vitamin D. Study has also concluded that vitamin D level maintenance reduces the risk of type 2 diabetes mellitus. Values of concentration of vitamin D, Ca^{+2} , P and PTH have been determined after respective assays and the values have been analyzed through analysis of variance (ANOVA). The mean $\pm$ SD of Vitamin D, Calcium, Phosphorous and Parathyroid hormone is 63.4492 ± 24.88 , 10.86 ± 0.99 , 1.552 ± 0.63 and ± 25.3 respectively. These results showed that the level of these micronutrients are very low in diabetic patients. So, there is need of awareness of vitamin D intake and fortified food.

P.154. EFFECT OF BIOPHYSIC TOOL (LASER TREATMENT) ON PRE-SOWING SEED OF *CUCURBITA MAXIMA* AND VARIATION IN THE YIELD THROUGH DIFFERENT BIOCHEMICAL AND ENZYMATIC ACTIVITIES RECORDED

Mazhar Abbas¹, Muhammad Arshad¹, Tariq Hussain², Zafar Hayat³ and Munawar Iqbal⁴

¹Department of Basic Sciences, Section Biochemistry, College of Veterinary and Animal Sciences Jhang, Sub Campus of University Veterinary and Animal Sciences Lahore, Pakistan.

²Department of Basic Sciences, Section Pharmacology and Toxicology, College of Veterinary and Animal Sciences Jhang, Sub Campus of University Veterinary and Animal Sciences Lahore, Pakistan.

³Department of Animal Sciences, College of Veterinary and Animal Sciences Jhang, Sub Campus of University Veterinary and Animal Sciences Lahore, Pakistan.

⁴Department of Chemistry, University of Lahore, Pakistan.

Corresponding author Email: mazhar.abbas@uvas.edu.pk

Cucurbita maxima belong to family *Cucurbitaceae*, it is widely used as a vegetable around the world. Different chemical and biochemical treatments were used to increase the production of different vegetables and fruits. In current study, pre sowing seed of *Cucurbita maxima* on embryonic side were treated with laser irradiation (He-Ne CW-laser 632.8 nm, density 1mW/cm², beam diameter 1 mm) for 3 and 5 minute. After 20 days of sowing, fresh tissue were collected and crude extract were prepared in organic solvent and change in different biochemical and enzymatic activities in control and treated group were recorded and effect of irradiation on treated group with respect to time were also documented. Laser treatment of *Cucurbita maxima* seeds enhanced the biochemical as well as the enzymatic activities. TPC (total phenolic contents), TFC (total flavonoids contents), TSS (total soluble sugar), reducing sugar, proline contents, total soluble protein and nitrogen contents were recorded higher in laser treated groups versus control. The pre-sowing seeds were treated with laser radiation for 3 and 5 min. TPC (total phenolic contents), TFC, (total flavonoids contents), proline contents, total soluble protein and nitrogenous compounds contents, ascorbic acid contents were recorded higher at 3 min. The laser irradiation effect on TSS (total soluble sugar), hydrogen peroxide (H₂O₂), malondialdehyde (MDA) was insignificant versus control. The SOD (superoxide dismutase) and POD (peroxidase), AMY (amylase), CAT (catalase) activities were recorded higher for 5 min laser treatment. Results revealed that He-Ne continuous wave-laser pre-sowing seed irradiation affected the seed coat mucilage, biochemical and enzymatic activities positively and this treatment could possibly be used to enhance the *Cucurbita maxima* productivity. Future study will be focused on growth at later stages and yield characteristics of *Cucurbita maxima*.

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