Pakistan Society for Biochemistry and Molecular Biology

15th Biennial International Conference

Trends in Molecular Sciences

April 14-17, 2025

UNIVERSITY OF THE PUNJAB
LAHORE-PAKISTAN

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Plenary Speakers + Invited Speakers

Prof. Ray Owens
University of Oxford, UK

Prof. William James
University of Oxford, UK

Dr. Mark William Corbett
University of York, UK

Prof. Keith Lindsey
Durham University, UK

Dr. Laura Lehtovirta-Morley
University of East Anglia, UK

Prof. Safwan Akram
Teesside University, UK

Dr. Kasra Esfahani
NIGEB, Iran

Prof. Fahrul Zaman Bin Huyop
Universiti Teknologi Malaysia

Prof. Sadaf Naz
SBS, PU, Lahore

Prof. Qurra-tul-Ann Afza Gardner
SBS, PU, Lahore

Prof. Samina Mehnaz
FCCU, Lahore

Prof. Naeem Rashid
SBS, PU, Lahore

Prof. Farzana Shaheen
ICCBS, Karachi

Prof. Abdul Qayyum Rao
CEMB, PU, Lahore

Prof. Zahra Hasan
AKU, Karachi

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Prof. Muhammad Ansar
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Prof. Saba Irshad
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Dr. Shazia Rafique
CEMB, PU, Lahore

Dr. Khurram Bashir
LUMS, Lahore

Prof. Tanveer Hussain
VU, Islamabad

Dr. Madeeha Afzal
University of Oxford, UK

Dr. Faiza Gul Durrani
Queen Mary University of London

Dr. Musharraf Jelani
ICP, Peshawar

Dr. M. Fayyaz ur Rehman
UOS, Sargodha

Dr. Safee Ullah Chaudhary
LUMS, Lahore

Dr. Muhammad Khan
PU, Lahore

Prof. Abdul Wadood
AWKUM, Mardan

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SBS, PU, Lahore

Dr. Khurram Joya
University of Madina, SA

Dr. Rahman Shah Zaib Saleem
LUMS, Lahore

Dr. Bushra Tabassum
SBS, PU, Lahore



Dr. Muhammad Irfan
UOS, Sargodha

Dr. Muhammad Saleem
SBS, PU, Lahore

Dr. Hafiz Muzzammel Rehman
SBB, PU, Lahore

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SBS, PU, Lahore

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SBS, PU, Lahore

Dr. Muhammad Farhan Ul Haque
SBS, PU, Lahore

Dr. Mohsin Shad
SBS, PU, Lahore

Time	Day-1, Monday 14 April 2025				Time	Day-2, Tuesday 15 April 2025				Time	Day-3, Wednesday 16 April 2025				Time	Day-4, Thursday 17 April 2025
08:00-09:30	Registration (LC)				09:00-11:00	Concurrent Scientific Sessions				09:00-11:00	Concurrent Scientific Sessions				09:00-10:30	Concluding Ceremony / Best Oral and Poster Awards
						UGB PE/P	SBS CB-1	SBB E/V	SC MR/MC		UGB MG-II	SBS AI	SBB PG/P	SC SC/RM		
09:30-11:00	Inaugural Session (LC)				09:00-09:25	IL-09	IL-10	IL-11	IL-12	09:00-09:25	IL-13	IL-15	IL-19	IL-21		
					09:25-09:40	OP-21	OP-28	OP-35	OP-42	09:25-09:50	IL-14		IL-20			
					09:40-09:55	OP-22	OP-29	OP-36	OP-43			IL-16		IL-22		
					09:55-10:10	OP-23	OP-30	OP-37	OP-44	09:50-10:05	OP-49		OP-54			
					10:10-10:25	OP-24	OP-31	OP-38	OP-45	10:05-10:20	OP-50	IL-17	OP-55	OP-59		
					10:25-10:40	OP-25	OP-32	OP-39	OP-46	10:20-10:35	OP-51		OP-56			
					10:40-10:55	OP-26	OP-33	OP-40	OP-47	10:35-10:50	OP-52	IL-18	OP-57	OP-60	11:00-13:00	PSBMB General Body Meeting
					10:55-11:10	OP-27	OP-34	OP-41	OP-48	10:50-11:05	OP-53		OP-58			
11:05-11:30	Tea / Refreshment (LCL) Poster Evaluation PP:1-66 (LCL)				11:10-11:30	Tea / Refreshment (LCL) Poster Evaluation PP:67-124 (LCL)				11:05-11:30	Tea / Refreshment (LCL)					
11:30-12:15	PL-1 (LC) Prof. Keith Lindsey				11:30-12:15	PL-4 (LC) Dr. Laura Lehtovirta-Morley				11:30-12:15	PL-7 (LC) Dr. Mark William Corbett					
12:15-13:00	PL-2 (LC) Prof. Sadaf Naz				12:15-13:00	PL-5 (LC) Prof. Fahrul Zaman Bin Huyop				12:15-13:00	PL-8 (LC) Prof. Safwan Akram					
13:00-14:00	Lunch/Prayer Break (LCL)				13:00-14:00	Lunch/Prayer Break (LCL)				13:00-14:00	Lunch/Prayer Break (LCL)					
14:00-14:45	PL-3 (LC) Prof. Ray Owens				14:00-16:00	Infectious Disease Symposia (LC) Sponsored By: PAS				14:00-14:45	PL-9 (LC) Dr. Kasra Esfahani					
15:00-17:05	Concurrent Scientific Sessions				14:00-14:30	SL01: Prof. Zahra Hasan				15:00-17:05	Concurrent Scientific Sessions				14:00-19:00	City Tour / Optional
	UGB AB/GE	SBS MG-I	SBB E/IB	SC EB/B	14:30-15:00	SL02: Prof. Waheed Akhtar					UGB BI/SB	SBS M/CB-11	SBB E/P	SC DD		
15:00-15:25	IL-01	IL-03	IL-05	IL-07						15:00-15:25	IL-23	IL-25	IL-26	IL-27		
15:25-15:50	IL-02	IL-04	IL-06	IL-08	15:00-15:30	SLO3: Dr Shazia Rafique				15:25-15:40	IL-24	OP-65	OP-71			
15:50-16:05	OP-01	OP-06	OP-11	OP-16						15:40-15:55		OP-66	OP-72	OP-77		
16:05-16:20	OP-02	OP-07	OP-12	OP-17	15:30-16:00	SLO4: Dr. Madeeha Afzal				15:55-16:10	OP-61	OP-67	OP-73	OP-78		
16:20-16:35	OP-03	OP-08	OP-13	OP-18						16:10-16:30	OP-62	OP-68	OP-74	OP-79		
16:35-16:50	OP-04	OP-09	OP-14	OP-19	16:00-16:50	PL-6 (LC) Prof. William James				16:30-16:45	OP-63	OP-69	OP-75	OP-80		
16:50-17:05	OP-05	OP-10	OP-15	OP-20						16:45-17:00	OP-64	OP-70	OP-76	OP-81		

PL: Plenary Lecture **IL:** Invited Lecture **OP:** Oral Presentation **PP:** Poster Presentation **SL:** Symposium Lecture
UGB: Undergraduate Building **SBS:** School of Biological Sciences **SBB:** School of Biochemistry & Biotechnology **SC:** School of Chemistry **LC:** Law College
PUECL: Punjab University Executive Club Lawns **LCL:** Law College Lawns * Abbreviation used below UGB, SBS, SBB & SC showing different concurrent sessions.

PROGRAM

Day-1 (Monday, April 14, 2025)

Inaugural Session (LC)	
Time	**Program**
08:00 – 09:30	Registration
09:00 – 09:30	Guests to be Seated
09:30 – 09:40	Recitation from the Holy Quran & National Anthem
09:40 – 09:50	Welcome remarks by Prof. Dr. M. Waheed Akhtar (Convener, PSBMB-TMS-2025)
09:50 – 10:10	Address by Prof. Dr. Khalid Mahmood Khan President, *Pakistan Society for Biochemistry and Molecular Biology*
10:10 – 10:30	Address by Prof. Dr. Kauser Abdullah Malik President, *Pakistan Academy of Sciences*
10:30 – 10:50	Address by Prof. Dr. Muhammad Ali Vice Chancellor, University of the Punjab, Lahore
10:50-10:55	Vote of Thanks (Dr. Muhammad Sajjad)
10:55 – 11:00	Group Photo
11:05 – 11:30	Tea/Refreshments (LC Lawn). Poster Evaluation PP:1-66 (LCL)
Plenary Session-I (LC) **Chairs: Prof. Dr. Khalid Mahmood Khan, Prof. Dr. M. Waheed Akhtar** **Moderators: Dr. Muhammad Sajjad, Mr. Qadeer Ahmad**	
11:30 – 12:15	**PL-01:**Prof. Muhammad Izazud Din Chughtai Memorial Lecture Regulation of stem cell maintenance in the Arabidopsis root meristem **Prof. Dr. Keith Lindsey,** Department of Biosciences, Durham University, UK
12:15 – 13:00	**PL-02:** Dr. Ata-ur-Rahman Memorial Lecture Research on epileptic disorders in Pakistan identifies multiple genetic variants and enables precision medicine for some patients **Prof. Dr. Sadaf Naz,** SBS, University of the Punjab, Lahore.
13:00 -14:00	**Prayer Break/Lunch (LC Lawns)**
14:00 -14:45	**PL-03:** Trimeric nanobodies potently neutralize Omicron variants of SARS-CoV-2 **Prof. Dr. Ray Owens,** Rosalind Franklin Institute, University of Oxford, UK.
Scientific Sessions-I (UGB) **Theme: Agriculture Biotechnology/Gene Editing for Crop Improvement** **Chairs: Prof. Dr. Keith Lindsey, Prof. Dr. Samina Mehnaz** **Moderator: Dr. Bushra Tabassum**	
15:00-15:25	**IL-01:** Transforming agriculture: next-generation crop resistance and genome editing breakthroughs at CEMB **Prof. Dr. Abdul Qayyum Rao,** CEMB, University of the Punjab, Lahore
15:25-15:50	**IL-02:** Characterization of metabolites of *Pseudomonas chlororaphis* and assessment of their potential as biocontrol agent **Prof. Dr. Samina Mehnaz,** Kauser Abdulla Malik School of Life Sciences, Forman Christian College University, Lahore.
15:50-16:05	**OP-01:** A biochemical and molecular docking insight towards bio-protection of rice plants against brown leaf spot disease by *Bacillus megaterium* strain Z-06. **Dr. Waheed Akram,** Department of Plant Pathology, University of the Punjab, Lahore.

16:05-16:20	**OP-02:** Exploring the genetic basis of the stay-green trait in bread wheat: a GWAS approach. **Dr. Sadia Latif,** Allama Iqbal Open University, Islamabad.
16:20-16:35	**OP-03:** CRISPR/Cas9 mediated mutagenesis of VISCOSITY 1 gene for tomato shelf-life enhancement. **Dr. Nazia Rehman,** National Agricultural Research Centre, Islamabad.
16:35-16:50	**OP-04:** Exploring correlations of key nutritional factors in different Pakistani wheat varieties. **Ms. Zulekha Zameer,** Forman Christian College (A Charted University).
16:50-17:05	**OP-05:** Harnessing CRISPR-Cas9 to remove β-Lactoglobulin: a path to allergen-free dairy. **Mr. Kaleem Ullah,** Bahauddin Zakariya University, Multan.
Scientific Sessions-II (SBS) **Theme: Medical Genetics-I** **Chairs: Prof. Dr. Sadaf Naz, Prof. Dr. Muhammad Ansar** **Moderator: Dr. Ayesha Imtiaz**	
15:00-15:25	**IL-03:** Genetic landscape of retinal dystrophies in Pakistani families. **Prof. Dr. Muhammad Ansar,** Department of Biochemistry, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad
15:25-15:50	**IL-04:** Identification of novel mutations in MCPH1 gene in a Pakistani family with microcephaly**.** **Prof. Dr. Saba Irshad,** SBB, University of the Punjab, Lahore.
15:50-16:05	**OP-06:** Expression analysis of ABCA1 in Type 2 diabetic Pakistani patients with and without dyslipidemia and correlation with glycemic index and lipid profile. **Dr. Amber Zaidi,** Army Medical College, National University of Medical Sciences (NUMS), Rawalpindi.
16:05-16:20	**OP-07:** Mitigation of NSAID-induced enteropathy through dietary Psyllium husk supplementation in a mouse model. **Dr. Soumble Zulfiqar,** SBS, University of the Punjab, Lahore.
16:20-16:35	**OP-08:** Identification of novel genetic variants underlying intellectual disability in Pakistani families. **Dr. Asif Mir,** International Islamic University, Islamabad.
16:35-16:50	**OP-09:** Mutation in Superoxide Dismutase Gene (SOD_2) and its relation with oxidative stress in Hypertension. **Dr. Riffat Iqbal,** Government College University Lahore.
16:50-17:05	**OP-10:** Novel sequence variants in the *SLC9A7* and *IQSEC2* genes in a family with X-linked intellectual disability. **Mr. Muhammad Ayaz,** Islamia College University Peshawar.
Scientific Sessions-III (SBB) **Theme: Enzymology & Industrial Biotechnology** **Chairs: Prof. Dr. Naeem Rashid, Dr. Muhammad Khurshid** **Moderator: Dr. M. Shahbaz Aslam**	
15:00-15:25	**IL-05:** Bioscience: Dry or wet? **Prof. Dr. Naeem Rashid,** SBS, University of the Punjab, Lahore.
15:25-15:50	**IL-06:** Production and characterization of recombinant enzymes for the poultry feed industry **Prof. Dr. Muhammad Tayyab,** UVAS, Lahore.

15:50-16:05	**OP-11:** The role of enzymes in biochemical catalysis advances and its applications **Dr. Shiv Ram Ashraf,** University of Gujrat, Gujrat.
16:05-16:20	**OP-12:** Unlocking the potential of *Thermogutta terrifontis*: t-end5a, a multifunctional enzyme for the bioethanol industry **Dr. Naveed Hussain,** SBB, University of the Punjab, Lahore.
16:20-16:35	**OP-13:** Industrial potential of recombinant manganese-catalases from *Geobacillus thermopakistaniensis* for sustainable textile bleach clean-up **Dr. Abeera Shaeer,** SBS, University of the Punjab, Lahore.
16:35-16:50	**OP-14:** Bioconversion of starchy food waste into maltose and glucose syrups using *Thermococcus kodakarensis* pullulan hydrolase **Dr. Majida Atta Muhammad,** SBS, University of the Punjab, Lahore.
16:50-17:05	**OP-15:** Pcal_0976, a pullulanase homologue from *Pyrobaculum calidifontis*, shows unique intragenomic evolution and substrate specificity **Dr. Iqra Aroob,** University of Child Health Sciences, Lahore.
Scientific Sessions-IV (SC) **Theme: Environmental Biology & Bioremediation** **Chairs: Prof. Dr. Fahrul Zaman Bin Huyop, Dr. Laura Lehtovirta-Morley** **Moderator: Dr. Mehwish Aslam**	
15:00-15:25	**IL-07:** Biotransformation of agro-industrial wastes to enzymes through fermentation **Dr. Muhammad Irfan,** Department of Biotechnology, University of Sargodha.
15:25-15:50	**IL-08:** Biosynthesis and immobilization of lignocellulosic byproducts derived protease as a robust biocatalytic system for various industrial applications **Prof. Dr. Tahir Mehmood,** MMG**,** University of the Punjab, Lahore.
15:50-16:05	**OP-16:** From waste to value: valorization of food waste through enzymatic processing **Dr. Nasir Ahmad,** SBS, University of the Punjab, Lahore.
16:05-16:20	**OP-17:** A track for the district Sialkot, Pakistan, to achieve the World Health Organization's HCV elimination plan of 2030 **Dr. Muhammad Rashid,** Grand Asian University Sialkot.
16:20-16:35	**OP-18:** Nitrogen-dependent diversity dynamics of cultivable methanol-utilizing bacterial community in rice paddy bulk and rhizospheric soils **Dr. Tabassum Yousaf,** SBS, University of the Punjab, Lahore.
16:35-16:50	**OP-19:** Development of microbial organic fertilizer using pilot-scale solid-state fermenter **Prof. Dr. Farooq Latif,** Minhaj University Lahore.
16:50-17:05	**OP-20:** Bacterial cell-based bioremediation of industrial wastewater: isolation and characterization **Dr. Sumaira Mehboob,** School of Biochemistry, Minhaj University Lahore.

Day-2 (Tuesday, April 15, 2025)

Scientific Sessions-V (UGB) **Theme: Protein Engineering & Proteomics** **Chairs: Prof. Dr. Naeem Rashid, Prof. Dr. Qurratulann Afza Gardner** **Moderator: Dr. Mehwish Aslam**	
09:00-09:25	**IL-09:** Engineering of insulin derivatives **Prof. Dr. Qurratulann Afza Gardner,** SBS, University of the Punjab, Lahore.
09:25-09:40	**OP-21:** Enhancing solubility and stability of *Pyrococcus abyssi* amylase-catalytic domain through sumo fusion for industrial applications. **Dr. Mohsin Shad,** SBS, University of the Punjab, Lahore.
09:40-09:55	**OP-22:** Mosquitocidal potential of a novel surface layer protein from *Geobacillus thermopakistaniensis* MAS1. **Dr. Hamayun Arshad,** SBS, University of the Punjab, Lahore.
09:55-10:10	**OP-23:** Genetic engineering and characterization of human serum amyloid A (SAA). **Ms. Areeba Shehzadi,** SBS, University of the Punjab, Lahore.
10:10-10:25	**OP-24:** Probing the functional significance of t263 residue in the regulatory site of recombinant L-asparaginase: *AfASNase.* **Dr. Beenish Maqsood,** SBB, University of the Punjab, Lahore.
10:25-10:40	**OP-25:** Insights into the differential proteome landscape of a newly isolated *Paramecium* species in response to metal stress. **Dr. Itrat Zahra**, University of the Punjab, Lahore.
10:40-10:55	**OP-26:** Molecular construction and engineering of a *Pyrococcus abyssi*-sourced lysophospholipase for enhanced stability and activity. **Dr. Arshia Nazir,** SBS, University of the Punjab, Lahore.
10:55-11:10	**OP-27:** Recombinant production of a thermostable pectinase: challenges and strategies **Ms. Aqsa Anwar,** SBS, University of the Punjab, Lahore.
Scientific Sessions-VI (SBS) **Theme: Cancer Biology-I** **Chairs: Prof. Dr. M. Safwan Akram, Prof. Dr. Farzana Shaheen** **Moderator: Dr. Muhammad Akhtar Ali**	
09:00-09:25	**IL-10:** Development of intranasal formulation of Z-acid as neuroprotective agent and Temporin SHa analogs as new anticancer peptides. **Prof. Dr. Farzana Shaheen,** ICCBS, University of Karachi, Karachi.
09:25-09:40	**OP-28:** Structural insights into RAL GTPase membrane dynamics and their role in colorectal cancer progression. **Dr. Arooj Shafiq,** Aga Khan University, Karachi.
09:40-09:55	**OP-29:** Advancing theranostic nanomedicines for targeted therapy in gastrointestinal cancer **Dr. Madeeha Shahzad Lodhi,** Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore.
09:55-10:10	**OP-30:** Tracking molecular response of tyrosine kinase inhibitors therapy in chronic myeloid leukemia patients using RT-PCR profiling of BCR-ABL transcripts **Dr. Amina Arif,** University of Central Punjab, Lahore**.**
10:10-10:25	**OP-31:** Unveiling the anticancer potential of antarctic yeast extracts against ovarian cancer cells **Ms. Maheen Fatima,** University of the Punjab, Lahore.

10:25-10:40	**OP-32:** Effect of palmitate treatment on doxorubicin response of breast cancer cell line **Ms. Muqadas,** SBS, University of the Punjab, Lahore.
10:40-10:55	**OP-33:** Harnessing the therapeutic potential of incensole acetate terpene nanoemulsion in targeting breast cancer: a translational approach toward therapeutic strategies **Ms. Iffat Nayila,** The University of Lahore, Lahore.
10:55-11:10	**OP-34:** Evaluating sotorasib and adagrasib against docetaxel in advanced non-small cell lung cancer treatment: a systematic review and meta-analysis **Ms. Safa Rafique,** University of the Punjab, Lahore.
Scientific Sessions-VII (SBB) **Theme: Immunology & Vaccinology** **Chairs: Dr. Muhammad Khurshid, Dr. Muhammad Saleem** **Moderator: Dr. M. Shahbaz Aslam**	
09:00-09:25	**IL-11:** Structural, functional and immunological characterization of an outer membrane protein, FrpBDr **Dr. Muhammad Saleem,** SBS, University of the Punjab, Lahore.
09:25-09:40	**OP-35:** Immuno-informatics-based prediction of putative T-cell epitopes for pancreatic cancer vaccine **Dr. Saba Zafar,** Department of Biochemistry and Biotechnology, The Women University, Multan.
09:40-09:55	**OP-36:** Advanced molecular diagnosis and prevalence of multi-drug resistance and extensive drug resistance tuberculosis with GeneXpert **Mr. Hafiz Anas Saeed,** Allama Iqbal Medical College, Lahore.
09:55-10:10	**OP-37:** Production and characterization of adenovirus-based VP2 viral vector vaccine against local isolates causing infectious bursal disease in chickens **Ms. Andleeb Aslam,** SBS, University of the Punjab, Lahore.
10:10-10:25	**OP-38:** An *in-vivo* experimental validation of the predicted T-cell epitopes for respiratory syncytial virus (RSV) **Ms. Aiman Fatima,** CAMB, University of the Punjab Lahore
10:25-10:40	**OP-39:** Prevalence of thyroid autoimmunity in HCV patients despite normal thyroid function tests in Pakistan **Dr. Shan Elahi,** Centre for Nuclear Medicine, Mayo Hospital Lahore.
10:40-10:55	**OP-40:** RT-LAMP: Advancing HCV variant detection beyond commercial RT-qPCR **Ms. Zilwa Mumtaz,** SBS & Forman Christian College University, Lahore.
10:55-11:10	**OP-41:** Diagnostic potential of GFAP, TNF- α and IL-6 in stroke subtypes and severity **Dr. Madeeha Mushtaq,** University of Health Sciences, Lahore.
Scientific Sessions-VIII (SC) **Theme: Anti-Microbial Resistance & Medicinal Chemistry** **Chairs: Prof. Dr. Abid Azhar, Prof. Dr. Tahir Mehmood** **Moderator: Dr. Soumble Zulfiqar**	
09:00-09:25	**IL-12:** Virulence and antibiotic resistance modulation by Paerucumarin in *Pseudomonas aeruginosa* **Dr. Uzma Qaisar,** SBS, University of the Punjab, Lahore.
09:25-09:40	**OP-42:** Biofilm-forming potential and occurrence of hospital-associated marker (*is16* gene) among *Enterococcus faecalis* **Dr. Hassan Bin Asif,** Al-Tibri Medical College, Isra University Karachi Campus, Karachi.

09:40-09:55	**OP-43:** High frequency and molecular characterization of ESBLs and Carbapenemases producing gram-negative bacterial isolates from burn patients **Dr. Muhammad Hayat Haider,** IMMG, University of the Punjab, Lahore.
09:55-10:10	**OP-44:** Whole genome sequencing-based analysis of antimicrobial resistance and virulence factors in *Salmonella gallinarum* isolated from poultry **Dr. Adnan Mehmood,** Department of Microbiology, Gulab Devi Educational Complex, Lahore.
10:10-10:25	**OP-45:** Novel antibacterial activity of nitro group in small-molecule sulphonamides against antibiotic-resistant uropathogenic *E. coli* **Ms. Farah Ashraf,** Lahore University of Management Sciences, Lahore.
10:25-10:40	**OP-46:** *In-vitro* efficacy of Meropenem-Vaborbactam against carbapenem-resistant *Pseudomonas aeruginosa* **Mr. Arslan Ullah Khan,** CAMB, University of the Punjab, Lahore.
10:40-10:55	**OP-47:** Assessment of the anticancer and antimicrobial potential of bioactive metabolites and optimization of culture conditions of *Pseudomonas aurantiaca* PB-St2 for high yields **Ms. Mahnoor Zameer,** FC College University, Lahore.
10:55-11:10	**OP-48:** Morphological Properties of Laser-Irradiated Biomaterials **Dr. Imtiazullah Khawaja,** Hazara University Mansehra, Pakistan.
11:10 – 11:30	**Tea/Refreshments (LC Lawn).** **Poster Evaluation PP:67-124 (LCL)**
Plenary Session II (LC) **Chairs: Dr. Mark William Corbett, Prof. Dr. Keith Lindsey** **Moderator: Dr. Muhammad Sajjad**	
11:30 – 12:15	**PL-04:** Substrate analogues as tools to study elusive enzymes in the global nitrogen cycle **Dr. Laura Lehtovirta-Morley**, University of East Anglia, UK.
12:15 – 13:00	**PL-05:** Harnessing halophilic bacteria for bioremediation of halogenated contaminants in hypersaline environments **Prof. Dr. Fahrul Zaman Bin Huyop**, University Technology, Malaysia.
13:00 -14:00	Prayer Break/Lunch (LC Lawns)
Scientific Sessions: Infectious Disease Symposium (LC). **Sponsored by: Pakistan Academy of Sciences.** **Chairs: Prof. Dr. Fahrul Zaman Bin Huyop, Prof. Dr. M. Waheed Akhtar** **Moderator: Dr. Muhammad Sajjad, Mr. Qadeer Ahmad**	
14:30-15:00	**SL-01:** Investigating host immunity to SARS-COV-2 variants through the pandemic in the context of different COVID-19 vaccinations administered in Pakistan **Prof. Dr. Zahra Hasan,** Aga Khan University, Karachi.
15:00-15:30	**SL-02:** Designing successful serodiagnosis for TB based on the selected antigens **Prof. Dr. M. Waheed Akhtar**, SBS, University of the Punjab, Lahore
15:30-16:00	**SL-03:** Understanding the epigenetic impact of hepatitis C virus: insights into insulin resistance as an emerging threat **Dr. Shazia Rafique,** CEMB, University of the Punjab, Lahore.
16:00-16:30	**SL-04:** Determination of resilience of a panel of broadly neutralising mAbs to emerging variants of SARS-CoV-2 generated using reverse genetics **Dr. Madeeha Afzal,** University of Oxford, UK
16:30-17:30	**PL-06:** Pandemic prevention: The role of basic science, public policy and biotechnology in COVID-19 and beyond **Prof. Dr. William James,** University of Oxford, United Kingdom.
17:30-18:00	**Tea (LCL)**

Day-3 (Wednesday, April 16, 2025)

Scientific Sessions-IX (UGB) **Theme: Medical Genetics-II** **Chairs: Prof. Dr. Sadaf Naz, Prof. Dr. Saima Saleem** **Moderator: Dr. Soumble Zulfiqar**	
09:00-09:25	**IL-13:** Next-generation sequencing is a compulsion and not an option in molecular diagnostics **Dr. Musharraf Jelani,** Centre for Omics Sciences, Islamia College, Peshawar.
09:25-09:50	**IL-14:** London-Pakistan Parkinson project **Dr. Faiza Gul Durrani,** Queen Mary University of London, UK.
09:50-10:05	**OP-49:** Polygenic risk score (PRS) analysis of genetic variants in a pediatric Pakistani population with ventricular septal defects (VSDs) **Dr. Shabana,** University of the Punjab, Lahore.
10:05-10:20	**OP-50:** Identification of novel missense pathogenic variant in FGFR3 gene causing achondroplasia (ACH) in Pakistani patients **Dr. Asia Parveen,** Gulab Devi Educational Complex, Lahore.
10:20-10:35	**OP-51:** Whole genome sequencing of Lumpy Skin Disease Virus (LSDV) in indigenous breeds of cattle: a step forward for prevention and treatment **Dr. Roohi Kanwal,** KIBGE, University of Karachi, Karachi.
10:35-10:50	**OP-52:** A molecular genetic association study of prothrombin (F2) gene rs1799963 G/A polymorphism and its role in atherosclerosis. **Mr. Farrakh Ali Abbas,** Department of Biotechnology, University of Sargodha.
10:50-11:05	**OP-53:** Whole genome amplicon sequencing of dengue and SARS-CoV-2 viral genomes using Oxford Nanopore Technologies **Mr. Ibrar Ahmed,** Alpha Genomics & Korea Research Institute of Standards and Science.
Scientific Sessions-X (SBS) **Theme: Artificial Intelligence in Life Sciences** **Chairs: Prof. Dr. Abdul Wadood, Dr. Laura Lehtovirta-Morley** **Moderator: Dr. Naseema Azim**	
09:00-09:35	**IL-15:** Exploring artificial neural networks and nano informatics for *bacillus cereus*-based synthesis of cysteine-conjugated bimetallic nanoparticles for diesel degradation **Dr. Muhammad Naveed,** University of Central Punjab, Lahore.
09:35-10:05	**IL-16:** Redefining molecular boundaries: the AI renaissance in protein structure and drug development **Dr. M. Fayyaz ur Rehman**, University of Sargodha.
10:05-10:35	**IL-17:** Statistical mechanics approaches to develop combinatorial cancer therapeutics **Dr. Safee Ullah Chaudhary,** Lahore University of Management Sciences, Lahore.
10:35-11:05	**IL-18:** Artificial Photosynthesis and Artificial intelligence (AI): Efficient conversion of solar energy and water into Fuels **Dr. Khurram Joya,** International Islamic University of Madinah, Saudi Arabia.
Scientific Sessions-XI (SBB) **Theme: Plant Genomics & Pathology** **Chairs: Prof. Dr. Keith Lindsey, Prof. Dr. Abdul Qayyum Rao** **Moderator: Dr. M. Shahbaz Aslam**	
09:00-09:25	**IL-19:** Regulating plant metabolism for stress resilience **Dr. Khurram Bashir,** Lahore University of Management Sciences, Lahore.

09:25-09:50	**IL-20:** Unravelling RXLR effector proteins in transgenic potato: A key to enhancing protection against late blight disease pathogenesis **Dr. Bushra Tabassum,** SBS, University of the Punjab, Lahore.
09:50-10:05	**OP-54:** Expression of MoSIGIII triggers unprecedented enhancement in sugar contents in transgenic sugarcane **Dr. Mudassar Fareed Awan,** University of Management and Technology, Sialkot.
10:05-10:20	**OP-55:** Biocontrol of *Fusarium wilt* in bell pepper using rhizospheric *Bacillus* strains with multifunctional plant growth-promoting traits **Ms. Ayesha Ishtiaq,** Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore.
10:20-10:35	**OP-56:** Isolation and characterization of microbial communities from the root of *Cicer arietinum* (Chickpeas) **Mr. Hamid Muzmmal Khan,** University of Agriculture, Faisalabad.
10:35-10:50	**OP-57:** Genomic insights into phosphine resistance in *Trogoderma granarium* through high-throughput sequencing **Dr. Roohi Ijaz,** University of the Punjab, Lahore.
10:50-11:05	**OP-58:** Genotoxicity induced by artificial food colors and food preservatives in *Allium sativum* (Garlic) root tip cells **Mr. Wasim Abbas,** Government College University, Lahore
Scientific Sessions-XII (SC) **Theme: Stem Cell & Regenerative Medicine** **Chairs: Prof. Dr. Abid Azhar, Prof. Dr. Asmat Salim** **Moderator: Dr. Ayesha Imtiaz**	
09:00-09:35	**IL-21:** Cell-based cardiac regeneration: role of WnT pathway modulators **Prof. Dr. Asmat Salim,** ICCBS, University of Karachi.
09:35-10:05	**IL-22:** Stem cells priming augments the therapeutic potency of stem cells for wound repair **Dr. Azra Mehmood,** CEMB, University of the Punjab.
10:05-10:35	**OP-59:** Stem cell-based α-terpineol-loaded hydrogels as advanced temporal wound dressings **Ms. Fatima Jameel,** Dr. Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Karachi.
10:35-11:05	**OP-60:** Sodium percarbonate-chitosan-based hydrogels with sustained oxygen release potential stimulate angiogenesis and accelerate wound healing **Dr. Tayyba Sher Waris,** IRCBM COMSATS University Islamabad, Lahore Campus.
Plenary Session III (LC) **Chairs: Prof. Dr. M. Waheed Akhtar, Prof. Dr. Keith Lindsey** **Moderator: Dr. Muhammad Sajjad, Mr. Qadeer Ahmad**	
11:30 – 12:15	**PL-07:** Development of bio-based products and processes for a better future **Dr. Mark William Corbett,** Director, Biorenewables Development Centre, United Kingdom.
12:15 – 13:00	**PL-08:** Biomanufacturing for tomorrow, today and yesterday **Prof. Dr. Muhammad Safwan Akram,** Teesside University, United Kingdom.
13:00 -14:00	**Prayer Break/Lunch (LC Lawns)**
14:30-14:55	**PL-09:** Harnessing the regulatory potential of 5′ UTRs to enhancing gene expression in plants **Prof. Dr. Kasra Esfahani,** National Institute of Genetic Engineering and Biotechnology, Iran.

Scientific Sessions-XIII (UGB) **Theme: Bioinformatics & Structure Biology** **Chairs: Prof. Dr. Abdul Wadood, Dr. Muhammad Naveed** **Moderator: Dr. Naseema Azim**	
15:00-15:25	**IL-23:** Identification of new and potent inhibitors for DHFR as a chemotherapeutic agent against cancer using machine learning-based virtual screening and *in vitro* assays **Prof. Dr. Abdul Wadood,** Abdul Wali Khan University Mardan, Pakistan.
15:25-15:55	**IL-24:** Identification of NS2B-NS3 protease inhibitors for therapeutic application in ZIKV infection: a pharmacophore-based high-throughput virtual screening and molecular dynamics simulations approach **Dr. Hafiz Muzzammel Rehman,** SBB, University of the Punjab, Lahore.
15:55-16:10	**OP-61:** Interaction between *Gossypium hirsutum* calmodulin-like protein 11 (CML11) and *Gemini virus*-encoded proteins using bioinformatics and molecular approaches **Dr. Aqsa Parvaiz,** The Women University Multan.
16:10-16:30	**OP-62:** Discovery of hydrazone derivatives as potent inhibitors of beta-secretase 1 for the management of Alzheimer's disease **Ms. Nehal Rana**, University of Central Punjab, Lahore.
16:30-16:45	**OP-63:** Genomic profiling of autism spectrum disorder in Pakistani children using bioinformatics tools **Mr. Farah Wazir,** COMSATS University Islamabad.
16:45-17:00	**OP-64:** Molecular recognition of salivary alpha-amylase via fullerene-porphin complexation in caries diagnosis **Dr. Sehrish Bilal,** Gulab Devi Educational Complex, Lahore.
Scientific Sessions-XIV (SBS) **Theme: Metabolomics & Cancer Genetics-II** **Chairs: Prof. Dr. Roquyya Gul, Prof. Dr. Asmat Salim** **Moderator: Dr. Muhammad Akhtar Ali**	
15:00-15:25	**IL-25:** Exploring synergistic interactions between natural bioactive molecules and arsenic trioxide in liver cancer: a novel approach to reduce systemic toxicity and improve therapeutic outcomes of arsenic trioxide **Dr. Muhammad Khan,** Institute of Zoology, University of the Punjab, Lahore.
15:25-15:40	**OP-65:** Cancer cell-type-dependent modifications of metastatic parameters by SLIT2-ROBO1 and RHOA camp signaling in response to TGFB1 and FGF2 **Dr. Quratulain Amjad,** Superior University Lahore.
15:40-15:55	**OP-66:** Anticancer effects of chrysin in promoting apoptosis in T47D tumor cells **Mr. Muhammad Sajid Hussain,** University of Okara, Punjab.
15:55-16:10	**OP-67:** Ligand-based drug design studies of different flavonoids as potential inhibitors of BCR-ABL **Mr. Muhammad Faisal Maqbool,** Institute of Zoology, University of the Punjab, Lahore.
16:10-16:30	**OP-68:** Alpha-2-Macroglobulin (A2M) and alpha-2-HS glycoprotein (FetuA), potential markers of renal cell carcinoma (RCC): an insight from the proteome profile of cancer tissues **Dr. Safa Akhtar,** The Women University, Multan.
16:30-16:45	**OP-69:** Nanoparticles-based delivery system for CRISPR-mediated gene disruption and to deliver CRISPR Cas9 components to cancer cells **Ms. Zainab Qaisar,** University of Central Punjab, Lahore.

16:45-17:00	**OP-70:** HDLBP contributes to goose fatty liver development by modulating oxidative stress and inflammatory responses (online) **Ms. Aneeqa Imtiaz,** Yangzhou University, Jiangsu Province, China.
Scientific Sessions-XV (SBB) **Theme: Epigenetics & Psychology** **Chairs: Prof. Dr. Saba Irshad, Prof. Dr. Tanveer Hussain** **Moderator: Dr. M. Shahbaz Aslam**	
15:00-15:25	**IL-26:** Genomic insights into yak (*Bos grunniens*): the keystone species for food security and sustainable livelihoods in northern Pakistan's changing climate **Prof. Dr. Tanveer Hussain,** Virtual University of Pakistan, Karachi.
15:25-15:40	**OP-71:** Epigenetic inactivation of *MST1* in tongue cancer: DNA methylation as a diagnostic and therapeutic target **Dr. Madiha Kanwal,** Department of Biosciences, Salim Habib University (SHU).
15:40-15:55	**OP-72:** Human blood and saliva DNA degradation associated with artificial ultraviolet and solar radiation as a function of exposure time **Ms. Kiran Bibi,** University of Swat, Swat.
15:55-16:10	**OP-73:** Beyond the trauma: epigenetics, PTSD, and the emergence of a reciprocal causality paradigm in psychiatric genetics **Ms. Aaitain Ikram Ul Haq,** University of the Punjab, Lahore.
16:10-16:30	**OP-74:** From possessiveness to peace: the psychology of Othello syndrome and the healing power of soul satisfaction **Ms. Mohammadu Yoosuf Fathima Askiya,** SBB, University of the Punjab, Lahore.
16:30-16:45	**OP-75:** Our skin biology predicts whether we are going to hell or heaven: a scientific link between our spiritual and physical existence **Dr. Shahzada Nadeem Abbas,** International Atlantic Virtual University.
16:45-17:00	**OP-76:** Nutrigenetics: Exploring the Genetic Influence on Vitamin D Levels in Pregnant Women of Pakistan **Dr. Shaheena Anwar,** Department of Biosciences, Salim Habib University (SHU).
Scientific Sessions-XVI (SC) **Theme: Drug Discovery** **Chairs: Prof. Dr. Qurratulann Afza Gardner, Dr. M. Fayyaz ur Rehman,** **Moderator: Dr. Ayesha Imtiaz**	
15:00-15:40	**IL-27:** Novel hit discovery for dengue virus (DENV) RNA-dependent RNA polymerase (RdRp) **Dr. Rahman Shah Zaib Saleem**, Lahore University of Management Sciences, Lahore.
15:40-15:55	**OP-77:** Antiadipogenic Potential of *Cissus quadrangularis* **Ms. Qindeel Fatima**, SBS, University of the Punjab, Lahore.
15:55-16:10	**OP-78:** Biofabricated AgZnO nanoparticles from C*itrus limon* peel extract: assessment of antioxidant, antimicrobial, and wound healing activities **Dr. Farah Deeba,** The Women University, Multan.
16:10-16:30	**OP-79:** Anti-convulsant activity of 3h-quinazoline-4-one derivatives: design, synthesis, and biological evaluation **Mr. Abu Sulman,** Department of Pharmacy University of Agriculture Faisalabad
16:30-16:45	**OP-80:** *In-silico* drug discovery of 2-aminobenzothiazole for anti-ulcer activity: a computational and pharmacological approach **Mr. Hafiz Aamir Ali Kharl,** Department of Pharmacy, University of Agriculture, Faisalabad.

16:45-17:00	**OP-81:** New derivatives of ibuprofen as potential LOX inhibitors; synthesis, characterization, in *vitro* and *in silico* studies **Mr. Lateef Ullah,** University of Central Punjab, Lahore**.**

Day-4 (Thursday, April 17, 2025)

09:00-10:30	**Concluding Ceremony / Best Oral and Poster Awards (LC)**
11:00-13:00	**PSBMB General Body Meeting**
14:00-19:00	**City Tour / Optional**

LC: Law College; **UGB:** Undergraduate Building; **SBS:** School of Biological Sciences; **SBB:** School of Biochemistry & Biotechnology; **SC:** School of Chemistry; **PL:** Plenary Lecture; **SL:** Symposium Lecture; **IL:** Invited Lecture; **OP:** Oral Presentation; **PP:** Poster Presentation; **PUECL:** Punjab University Executive Club Lawns; **LCL:** Law College Lawns.

Poster Presentation

Moderators:

Dr. Asima Tayyeb, University of the Punjab, Lahore

Dr. Sumera Zaib, University of Central Punjab, Lahore

Agriculture Biotechnology / Gene Editing for Crop Improvement	
PP: 01	Integrated genome-based breeding for developing climate-resilient crops in rapid time **Dr. Muhammad Ramzan Khan**, National Agricultural Research Centre, Islamabad.
PP: 02	Mitigation of drought stress by foliar spray of ZnO nanoparticles in wheat (*Triticum aestivum l*) **Ms. Tehreem Ghafoor,** Institute of Botany, Bahauddin Zakariya University, Multan.
PP: 03	Synthesis of nano-fertilizer by utilization of banana peel extract and its impacts on the growth of different plants **Ms. Sania Saleem,** University of Management and Technology, Lahore.
PP: 04	Enhancing Fe/Zn accumulation in wheat through optimized Fe/Zn fertilization strategy **Ms. Tayyaba Noor,** National Institute for Biotechnology & Genetic Engineering, Faisalabad.
PP: 05	Floral infusion biotechnological approach to sustainable fragrance. **Ms. Shehar Bano,** Gulab Devi Education Complex, Lahore.
PP: 06	Interaction analysis of *Ficus religiosa* plant with the *Begomovirus* **Ms. Laraib Tabassum,** University of Management and Technology, Lahore.
PP: 07	Phytochemical profiling and biological assessment of three plant species **Ms. Sitaish Justin Mall,** Kinnaird College Women University, Lahore.
PP: 08	Genome-wide identification and expression analysis of kinase proteins in land plants, with a focus on *Gossypium hirsutum* under abiotic stress **Ms. Sania Munir**, University of Management and Technology (UMT), Lahore.
Medical Genetics	
PP: 09	Detection of alpha-1-antitrypsin (*serpina1*) single nucleotide polymorphism (rs750766974) in COPD patients at Gulab Devi Teaching Hospital **Ms. Sidra Ishtiaq,** Gulab Devi Educational Complex, Lahore.
PP: 10	Assessment of human toll-like receptor 4 (tlr4) asp299gly (rs4986790) polymorphism on susceptibility of coronary artery disease **Ms. Zainab Nauman,** Gulab Devi Educational Complex, Lahore.
PP: 11	Association of leptin gene polymorphism with obesity and insulin resistance in Pakistani subjects **Ms. Sonaina Ehsan,** Gulab Devi Educational Complex Lahore.
PP: 12	Association of rs141502002, rs505151, rs777300852 and rs28362277 of pcsk9 gene polymorphisms with coronary artery disease in Pakistani population **Ms. Nireeta Yousaf** or Muhammad Osama Zafar, Gulab Devi Educational Complex, Lahore.
PP: 13	Association of SNP rs16969968 of CHRNA5 gene with COPD patients at Gulab Devi Teaching Hospital **Ms. Ifrah Asif,** Gulab Devi Educational Complex, Lahore.
PP: 14	Evaluation of oxidative stress and eryptosis induced by gemifloxacin and moxifloxacin in erythrocytes **Ms. Sana Akram,** University of Agriculture Faisalabad.
PP: 15	PCR-based diagnosis for *salmonella typhi* in clinical specimens **Ms. Hefza Ashraf,** University of Sargodha.
PP: 16	Impact of Shisha, cigarettes, and E-cigarettes on the Lungs of youngsters **Ms. Tehmina,** Minhaj University, Lahore.
PP: 17	Pharmacological management of pituitary macroadenoma: a non-surgical approach to tumor control and hormone regulation **Ms. Hafiza Amina Nadeem,** University of the Punjab Lahore.
PP: 18	Human DNA isolation and characterization from bedbugs (*Cimex lactolarius*) **Ms. Shahlila,** University of Swat.

PP: 19	Optimization of autosomal STR markers for equine genotyping using multiplex PCR **Mr. Usama Mustafa,** Centre for Applied Molecular Biology, University of the Punjab Lahore.
PP: 20	Euthanasia and self-assisted suicide **Ms. Wania Asif,** University of the Punjab Lahore.
PP: 21	Analysis of hotspot mutations of Ltbp2 Gene: A predominant driver of primary congenital glaucoma **Ms. Maha Munir,** Centre for Applied Molecular Biology, University of the Punjab, Lahore.
PP: 22	Pharmacogenetic perspective on clopidogrel: ABCB1 C3435T and CYP2C19*2 polymorphism in Swat Population **Ms. Bushra Khan,** University of Swat.
PP: 23	Synthesis of anti-interferon beta 1a antibody in mice and its characterization **Ms. Ayesha Murad,** University of Management and Technology, Lahore.
Enzymology & Industrial Biotechnology	
PP: 24	Optimization of submerged fermentation conditions for lipase production from *Bacillus mycoides* through response surface methodology **Mr. Moiz Ali,** Government Dyal Singh Graduate College Lahore.
PP: 25	Application of response surface methodology for optimizing the extracellular conditions for protease production from *Bacillus mycoides* **Ms. Rabia Asif,** Government Dyal Singh Graduate College Lahore.
PP: 26	Production, purification, and comparative insights on novel phytases from different bacterial strains **Ms. Hafiza Rifah Maryam Sultana,** University of the Punjab, Lahore.
PP: 27	Production and biochemical characterization of a recombinant catalase from *Clostridium difficile* **Ms. Qurat Ul Ain,** SBS, University of the Punjab, Lahore.
PP: 28	Assessment of banana peels as the substrate for the production of gallic acid via solid-state fermentation using response surface methodology **Ms. Shaina Zulnoor Fatima,** SBS, University of the Punjab, Lahore.
PP: 29	Purification and refolding of the SUMO-fused knob domain of fiber-2 protein of FOWL adenovirus-4 **Ms. Mahnoor Habib,** SBS, University of the Punjab, Lahore.
PP: 30	Molecular cloning, expression, and characterization of encapsulin-like hypothetical proteins from *Thermococcus kodakarensis* **Ms. Ayesha Zahid Ghuman,** SBS, University of the Punjab, Lahore.
PP: 31	Characterization of recombinant human serum amyloid p-component (SAP). **Ms. Iqra Riaz,** SBS, University of the Punjab, Lahore.
PP: 32	Pcal_0606: a hyperthermophilic phosphoglucose/phosphomannose isomerase with exceptional stability and activity **Ms. Amina Maqsood,** SBS, University of the Punjab, Lahore.
PP: 33	Optimization of culture conditions for the production of antibiotics by actinomycetes **Mr. Muhammad Taimoor Chishti,** Government College University, Lahore.
PP: 34	Molecular cloning, expression optimization and purification of a glycerophosphodiesterase from a hyperthermophilic archaeon *Pyrococcus abyssi* **Ms. Arooba Farman,** SBS, University of the Punjab, Lahore.
PP: 35	Mining and characterization of GTF1 from locally isolated novel species, *Apilactobacillus waqarii* **Ms. Hira Kiran,** SBS, University of the Punjab, Lahore.
PP: 36	Developing next-generation L-asparaginases in tackling therapeutic and industrial challenges **Ms. Ayesha Sania,** SBS, University of the Punjab, Lahore.
PP: 37	Machine learning-powered design and optimization of a novel bifunctional phytase-protease enzyme for enhanced phytic acid and protein utilization **Ms. Rida Naveed,** Kausar Abdullah Malik School of Life Sciences, Forman Christian College University, Lahore.
PP: 38	Production of recombinant DNA polymerase I from *Geobacillus thermopakistaniensis* for application in isothermal amplification techniques **Ms. Aqsa Anwar,** SBS, University of the Punjab, Lahore.

PP: 39	Evaluation of bacillus spp. as direct-fed microbials for poultry: enzyme production, biofilm formation, and antimicrobial activity **Ms. Sadia Ijaz,** Department of Biochemistry, University of Agriculture, Faisalabad.
PP: 40	In house production and characterization of plastic degrading enzymes **Mr. Usman Ramzan,** SBS, University of the Punjab, Lahore.
Drug Discovery	
PP: 41	Deciphering the role of honey in ocular diseases by integrating network pharmacology and molecular docking approaches for retinal therapy **Mr. Afaq Akram,** The University of Lahore.
PP: 42	Role of antiglycating agents to ameliorate AGES-induced cardiotoxicity **Ms. Sana Asad,** PCMD, ICCBS, University of Karachi.
PP: 43	Potential of antiglycation compounds against AGES-induced cardiomyocyte injury **Ms. Urooba Fatima,** PCMD, ICCBS, University of Karachi.
PP: 44	Bio-ceramics for drug delivery **Ms. Kainat Mehboob,** Gulab Devi Education Complex Lahore.
PP: 45	Valorization of hop shoots (*Humulus lupulus*) for their phenolic content and free radical scavenging activity **Dr. Muhammad Khalid Saeed,** PCSIR, Lahore.
PP: 46	Development of Kojic acid toner. **Ms. Momina Quddus,** Gulab Devi Education Complex Lahore.
PP: 47	Assessment of biological activities of secondary metabolites of *Pseudomonas aurantiaca* **Mr. Burhan Haider,** SBS, University of the Punjab, Lahore.
PP: 48	*In vitro* antioxidant, antimicrobial, and phytochemical analysis of ethanolic extract of *Brassica nigra* **Ms. Christina Arooj,** Gulab Devi Education Complex Lahore.
PP: 49	Nutritional miracles of *Moringa oleifera* for the betterment of human health **Ms. Tayyaba Asif,** Gulab Devi Education Complex Lahore.
PP: 50	Biopotential of purified bioactive compounds derived from *Citrullus colocynthis* **Ms. Aiman Aijaz,** Center of Excellence in Molecular Biology (CEMB), Punjab University, Lahore.
PP: 51	In-silico vaccine and drug designing against MAPK protein express for Alzheimer's disease in a zebrafish model **Ms. Ayesha Abbas,** National University of Sciences and Technology (NUST), Islamabad.
PP: 52	The biochemical paradigm of hope: a medical science analysis of logotherapy's therapeutic efficacy in modulating neurotransmitter systems **Mr. Muhammad Saad Faiz,** SBB, University of the Punjab, Lahore.
PP: 53	Synthesis and characterization of magnetic nanoparticles conjugated with methotrexate and folate for estimation of anti-bacterial and anti-cancer activity **Ms. Shagufta Malik,** Department of Biochemistry, University of Agriculture Faisalabad.
PP: 54	Novel amide derivatives as efflux pump inhibitors: a promising strategy for tackling multi-drug resistance in *Pseudomonas aeruginosa* **Dr. Muhammad Nazir Uddin,** University of Swat.
PP: 55	Chitosan-based hydrogels for pH-responsive and controlled release of vitamin D3 **Ms. Manahal Furqan,** Lahore College For Women University, Lahore.
PP: 56	A review on the development of bio-based vitamin C serum **Ms. Maha Asif,** Department of Biotechnology, Gulab Devi Educational Complex, Lahore.
PP: 57	BioHydraGel: Revolutionizing skincare through biotechnology **Ms. Zarish Fatima,** Department of Biotechnology, Gulab Devi Educational Complex, Lahore.
PP: 58	Production of a recombinant thrombolytic agent using streptokinase and tissue-type plasminogen activator **Mr. Junaid Hassan,** Al-Aleem Center for Advance Studies and Research, University of Lahore.

PP: 59	Synthesis of copper oxide deposited covalent triazine frame work for the colorimetric sensing of uric acid **Mr. Shahzil Mehmood,** Kohat University of Science and Technology.
Protein Engineering & Proteomics	
PP: 60	Structural and functional insights into *Bacillus licheniformis* α-amylase variants through protein engineering strategies **Dr. Mohsin Shad,** SBS, University of the Punjab, Lahore.
PP: 61	Engineering the laccase-xylanase chimera from *Bacillus subtilis* strain R5. **Mr. Ali Munir,** SBS, University of the Punjab, Lahore.
PP: 62	A comparative study of native and engineered xylanase from *Bacillus subtilis* strain R5 **Ms. Faiza Mehmood,** SBS, University of the Punjab, Lahore.
PP: 63	Engineering thermostable L-asparaginase for enhanced acrylamide mitigation. **Ms. Hira Akram,** SBS, University of the Punjab, Lahore.
PP: 64	*In vitro* half-life of human alpha-2b interferon derivative (PHE-IFN-B5) in plasma using ELISA **Ms. Amna Kainaat,** SBS, University of the Punjab, Lahore.
PP: 65	Identification of prognostic biomarkers in breast cancer using multi-omics data mining **Ms. Saeeda Tariq,** Centre of Excellence in Molecular Biology, University of the Punjab, Lahore.
PP: 66	Discovery and design of antimicrobial peptides (AMPS) for potential lung cancer therapy using multi-omics and machine learning **Ms. Iqra Mubeen,** Quaid-e-Azam University, Islamabad.
Cancer Biology & Metabolomics	
PP: 67	Use of HER2 to treat breast cancer **Ms. Isna Naeem,** Gulab Devi Educational Complex, Lahore.
PP: 68	Synthesis of copper oxide nanoparticles conjugated with cytarabine and estimation of its anticancer activity **Ms. Zomah Malik,** University of Management and Technology, Lahore.
PP: 69	Anti-cancer potential of *R. stricta* and its effect on de-novo lipid biosynthesis pathway in MCF-7 breast cancer cell line **Ms. Muqadas,** University of the Punjab, Lahore.
PP: 70	Role of citrate carriers (SLC25A1) in tumor progression **Ms. Mehak Shahid,** SBS, University of the Punjab, Lahore.
PP: 71	Enhancing anticancer efficacy through synergistic action of zinc nanoparticles and walnut extract **Ms. Memoona Suleman,** University of Central Punjab, Lahore.
PP: 72	Genetic identification, structural characterization and expression analysis of KPCQ in breast cancer **Ms. Mahnoor Basit,** National University of Sciences and Technology (NUST), Islamabad.
PP: 73	Unveiling the anticancer potential of antarctic yeast used in cancer treatment **Ms. Maheen Fatima,** University of the Punjab, Lahore.
Stem Cells & Regenerative Medicine	
PP: 74	Chicken-derived collagen for enhanced wound healing **Ms. Hunza Rashid,** Government College University, Faisalabad.
PP: 75	Propolis-mediated silver nanoparticles for wound healing and skin regeneration **Ms. Hadia Haq,** Lahore College for Women University, Lahore.
PP: 76	Curative effect of *aloe Barbadensis miller* in excisional wound healing mechanism using a rat model **Mr. Imran Haider,** Gulab Devi Educational Complex, Lahore.
PP: 77	Lymph node-based organ regeneration: developments, challenges, and future directions **Ms. Urooj Fatimah,** University of Management and Technology, Lahore.
PP: 78	Synthesis and characterization of novel fenugreek-infused hydrogels **Ms. Farhat Rafiq,** SBS, University of the Punjab, Lahore.
PP: 79	Combining mesenchymal stem cells, 3D scaffold, and small molecule: a tissue engineering approach for cardiovascular therapeutics **Dr. Rida-e-Maria Qazi,** (ICCBS), University of Karachi, Karachi.

PP: 80	Bioinspired Angiogenic and Anti-Inflammatory Apatite-Infused Self-Healing Hydrogels for Enhanced Bone Defect Repair **Ms. Aqsa Afzaal,** IRCBM, COMSATS University Islamabad, Lahore Campus, Pakistan.
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PP: 82	Production of bioplastics using recombinant bacteria **Ms. Ayesha Naveed,** Gulab Devi Educational Complex, Lahore.
PP: 83	Purification of wastewater to eradicate food scarcity. **Ms. Eeman Ali,** SBS, University of the Punjab, Lahore.
PP: 84	Simulated field trial of cemalgatech-1: a self-sustaining mosquito larvicidal transgenic microalga expressing *cry11ba* protein **Ms. Khadija Rani,** CEMB, University of the Punjab, Lahore.
PP: 85	Construction of an enzyme-based electrochemical biosensor using $NiCr_2O_4$/G-C_3N_4-modified pencil graphite electrode to investigate malathion sensitivity in insects **Mr. Syed Badar Zaidi,** Gulaab Devi Educational Complex, Lahore.
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PP: 87	Whole genome sequencing and metabolic pathway analysis of methylobacterium TP4: a novel methanol-utilizing phyllosphere bacterium **Ms. Sahar Andleeb,** SBS, University of the Punjab, Lahore.
PP: 88	Unveiling the power of TiO dopped ZnO nanomaterial as an effective solution in the leather industry **Ms. Eqra Farooq,** University of the Punjab, Lahore.
PP: 89	Production of polyhydroxyalkanoates (PHAS) by *Bacillus subtilis* from cellulase treated corncob Hydrolysate **Mr. Zubair Akram,** SBS, University of the Punjab, Lahore
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PP: 91	Development of indigenous human adenovirus 5-based vector vaccines against SARS-COV-2 and its emerging variants **Ms. Sofia Irfan,** SBS, University of the Punjab, Lahore.
PP: 92	Expression, purification, and structural characterization of recombinant receptor binding domain of SARS-COV-2 spike glycoprotein produced in Schneider 2 cells **Ms. Ayesha Siddiqa,** SBS, University of the Punjab, Lahore.
PP: 93	Biological application of *Artemisia vulgaris* leaves methanolic extract against ethanol-induced NLRP3 inflammasome in mice **Ms. Abida Perveen,** Department of Chemistry, Kohat University of Science and Technology, Kohat.
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PP: 95	Molecular insights into *hunchback* mRNA regulation in *drosophila*: a mass spectrometry-based approach **Ms. Saadia Qamar,** Lahore University of Biological and Applied Sciences, Lahore.
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PP: 98	Application of bioinformatics tools and software for comparative analysis of various cytochrome p450 **Mr. Abdullah Saeed,** Government Dyal Singh Graduate College Lahore.
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PP: 103	Determination of chemical constituents of *Melaleuca alternifolia* oil and their in-silico interaction with obp1 of *Aedes aegypti* **Ms. Zunaira Javaid,** Kinnaird College for Women, Lahore.
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PP: 111	Fusion of RV3874 with RV2031C from *mycobacterium tuberculosis* results in a two-fold increase in serodiagnostic potential **Dr. Nasir Mahmood,** SBS, University of the Punjab, Lahore.
PP: 112	Investigating sars-cov-2 infection in the post-pandemic period: a study of viral genomic sequences **Ms. Amina Ajmal,** Mohammad Ali Jinnah University, Karachi.
PP: 113	In vitro evaluation of the therapeutic efficacy of *Syzygium cumini* and *Trachyspermum ammi* against *Eimeria zuernii* coccidiosis in cattle **Ms. Arooj Rafique,** Gulab Devi Educational Complex, Lahore.
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PP: 115	Analysis of mutations in the receptor binding domain of SARS-CoV-2 in Borno state, Nigeria, and its effect on protein dynamics **Mr. Dayyabu Shehu,** Bayero University, Kano, Nigeria.

PP: 116	Antiplasmodial activity of *Azanza garckeana* stem bark extract and its effect on haematological indices in mice **Mahmoud Suleiman Jada**, Modibbo Adama University Yola, Adamawa State, Nigeria.
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PP: 118	Comparative genomic insights into multi-drug resistant Uropathogenic *Escherichia coli* from Pakistan reveal resistance and virulence patterns **Ms. Zaara Ishaq,** National University of Sciences and Technology, Islamabad.
PP: 119	Isolation and characterization of *Staphylococcus epidermidis* from human skin and bacteriophage infecting them **Ms. Ansa Javed,** SBS, University of the Punjab, Lahore.
PP: 120	Phenotypic characterization of MBL enzymes in urinary tract infection-causing pathogens **Ms. Shehzeen Usman,** University of Management and Technology, Lahore.
PP: 121	Isolation and purification of bacteriophages against *Mycobacterium fortuitum* **Ms. Shahzara,** Kinnaird College for Women University, Lahore.
PP: 122	Isolation and characterization of lytic bacteriophages against multidrug-resistant bacteria: a strategy to combat antimicrobial resistance **Ms. Zahra Haider,** CEMB, University of the Punjab, Lahore.
PP: 123	Antimicrobial potential of *Artemisia* spp. Extracts against multidrug-resistant *Shigella* and *Klebsiella* species **Ms. Zaveeba Shahid,** SBS, University of the Punjab, Lahore.
PP: 124	Conjugation of silver nanoparticles with mint, neem, and ginger extracts to undergo antimicrobial analysis **Ms. Aliza Mukhtar,** University of Management and Technology, Lahore

PL-01

Regulation of Stem Cell Maintenance in the Arabidopsis Root Meristem

Prof. Dr. Keith Lindsey*
Department of Biosciences, Durham University, Durham, UK.

ABSTRACT

The activity of meristems in central to the growth and development of plants. They are the sites of cell division and are maintained through the control of the identity and activity of stem cells within the meristems. These stem cells are undifferentiated but divide to generate surrounding differentiated tissues. We are interested in the molecular mechanisms that regulate stem cell identity and activity, with a particular focus on the root apical meristem of the model plant Arabidopsis. Root development is a critical feature of plant adaptation to various environments, as it anchors plants in the soil, acts as a mediator of soil stresses, and facilitates interaction with microorganisms. I will discuss our recent progress in identifying new molecular regulators of meristem function and show how the mathematical modelling of gene-signalling networks can help us understand the complexities of the regulatory process.

PL-02

Research on Epileptic Disorders in Pakistan Identify Multiple Genetic Variants and Enable Precision Medicine for Some Patients

***Prof. Dr. Sadaf Naz*[1],** *Ambreen Kanwal[1], Rimsha Zulfiqar[1], Anum Shafique[1], Amina Iftikhar[2], Go Hun Seo[3]*
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ABSTRACT

Epilepsy is a dysfunction of the nervous system characterized by recurring seizures. Psychosis is diagnosed if patients suffer from hallucinations or delusions. Epilepsy and psychosis may occasionally co-occur. We conducted a study to determine the causes of epilepsy with or without psychosis in multiple families from Pakistan. Patients were recruited from a local hospital. Epilepsy was diagnosed by neurologists while accompanying psychotic symptoms were evaluated by psychiatrists and a clinical psychologist. Exome and Sanger sequencing were completed for x selected participants, and the data were filtered according to a standardized pipeline. All patients were born to unaffected consanguineous parents. Examinations confirmed the presence of epilepsy with or without psychosis in the patients and their absence in other participants. Exome data analyses identified variants in multiple genes which could be linked to the phenotypes of the patients for all but one participating family. The variants caused epilepsy in the respective patients, except for a novel biallelic variant in

CLN8 which segregated with both epilepsy and treatment-resistant psychosis. Molecular diagnosis enabled identification of specific medications for patients in two families for treatment of their seizures. This study extends the genetic spectrum of epilepsy and expands the *CLN8*-related phenotype to include severe treatment-resistant psychosis. It further emphasizes the utility of molecular diagnosis for providing better treatment options to some patients with epilepsy.

PL-03

Trimeric Nanobodies Potently Neutralize Omicron Variants of SARS-CoV-2

Prof. Dr. Ray Owens*
The Rosalind Franklin Institute & University of Oxford, England.

ABSTRACT

The Omicron strains of SARS-CoV-2 pose a significant challenge to the development of effective antibody-based treatments as immune-evasion has compromised most available immune therapeutics. Therefore, in the 'arms race' with the virus, there is a continuing need to identify new biologics for the prevention or treatment of SARS-CoV-2 infections. Single domain antibodies (nanobodies) have proved effective in neutralising SARS-CoV-2 viruses both in vitro and in animal models of COVID-19. By screening nanobody phage display libraries previously generated from llamas immunized with either the Wuhan or Beta spike proteins we have identified nanobodies that bind to the Omicron BA.1 spike protein. The structure and binding properties of these nanobodies have been characterised providing insight into their binding epitopes on the Omicron spike protein. Trimeric versions have been shown to neutralise Omicron variants of SARS-CoV-2 in vitro and the hamster model of COVID-19 following nasal administration. Thus, either alone or in combination, such nanobodies could serve as starting points for the development of new anti-viral immune therapeutics.

PL-04

Substrate Analogues as Tools to Study Elusive Enzymes in the Global Nitrogen Cycle

Dr. Laura Lehtovirta-Morley*
University of East Anglia, UK.

ABSTRACT

Ammonia oxidation is a crucial part of the global nitrogen cycle and of major importance to food security, climate change and wastewater treatment. Ammonia oxidation is carried out by specific microorganisms using the key enzyme ammonia monooxygenase. Despite its significance in both natural and engineered environment, little is known about the function of ammonia monooxygenase and this enzyme has never been successfully purified in its active form. In this presentation, I will discuss the progress made into understanding the ammonia monooxygenase using substrate analogues, which are compounds structurally like the physiological substrate ammonia. Using substrate

analogues, it has been possible to examine the substrate and inhibitor ranges of ammonia monooxygenases and understand how substrates interact with the enzyme. Furthermore, we have developed a novel click chemistry-based approach to fluorescently tag active monooxygenases and demonstrated the use of this methodology in both pure microbial cultures as well as in the environment. The results can be useful for resolving fundamental mechanistic questions about nitrogen cycling, and more broadly, these techniques could be applied for characterising enzymes that are not amenable to purification and characterisation using typical methods.

PL-05

Harnessing Halophilic Bacteria for Bioremediation of Halogenated Contaminants in Hypersaline Environments

Prof. Dr. Fahrul Zaman Bin Huyop*
University Technology, Malaysia.

ABSTRACT

Hypersaline environments, characterized by salt concentrations exceeding seawater levels, represent extreme ecosystems harboring unique microbial life. These environments, including salt lakes and coastal lagoons, are often vulnerable to contamination by recalcitrant halogenated organic compounds. This study investigates the bioremediation potential of halophilic bacteria from Lake Tuz, Turkey, focusing on their ability to degrade 2,2-dichloropropionic acid (2,2-DCP), a model halogenated contaminant. Metagenomic analysis of Lake Tuz revealed a microbial community dominated by Firmicutes (88%), followed by Fusobacteria (6%) and Proteobacteria (5%). A novel dehalogenase-producing bacterium, Pseudomonas halophila strain HX (KR071871), was isolated and demonstrated near-complete degradation (99.3%) of 20 mM 2,2-DCP under optimal conditions. The dehalogenase gene (dehHX) was characterized, revealing a Group I dehalogenase with 82% sequence identity to DehI. The halo-stable nature of DehHX is likely an evolutionary adaptation for function in hypersaline conditions. This research highlights the potential of halophilic bacteria for bioremediation of halogenated contaminants in saline environments. This work is a collaborative project between Ondokuz Mayis University, Turkey, and Universiti Teknologi Malaysia, funded by TUBITAK, Turkey.

Keywords: *Bioremediation, Dehalogenase, Halophilic Bacteria, Halogenated Compounds, Hypersaline Environments, Pseudomonas halophila HX, Lake Tuz.*

PL-06

Pandemic Prevention: The Role of Basic Science, Public Policy and Biotechnology in COVID-19 and Beyond

Prof. Dr. William James*
University of Oxford, United Kingdom.

ABSTRACT

Pandemics have shaped human history, claiming tens of millions of lives through waves of infection. Today, factors like high population density, global travel, and ecological disruption increase the risk of future high-consequence pandemics. Yet, the global response to COVID-19 offers hope that this ancient threat can be managed—if we learn from recent experiences. For the first time in history, we developed and deployed vaccines and therapeutics at unprecedented speed, averting over 10 million deaths in 2021 alone. This remarkable achievement was made possible through unprecedented collaboration among scientists, technologists, businesses, and policymakers across borders. As someone who contributed to these efforts, I witnessed firsthand the power of innovation and global cooperation. However, challenges remain, and we must embed these lessons into enduring improvements to transnational systems. In my lecture in Suzhou, I will explore how we can build on this progress to better prepare for and respond to future pandemics, ensuring a safer, more resilient world.

PL-07

Development of Bio-based Products and Processes for a Better Future

Dr. Mark William Corbett*
Director, Biorenewables Development Centre, United Kingdom.

ABSTRACT

There is an urgent need to accelerate the transition to clean and safe technologies that will enable prosperous and environmentally positive chemicals and materials industries. Bio-based products and processes will be one of the key technologies for reducing the environmental impact of chemicals industry, promising safer and more sustainable routes to both new and established chemistries in a post-petrochemical global economy. The use of low value and bio-based feedstocks promises to increase economic returns to producers of biomass and waste, whilst avoiding competition with food and energy production. These technologies can be implemented to take advantage of regionally available feedstocks, offering the shared benefits of addressing local waste streams and economic growth. The Biorenewables Development Centre is an open-access research, development and scale-up facility based in the North of England, supporting innovation that returns sustainable economic and environmental value across many industrial sectors. The Centre hosts a unique concentration of

expertise and facilities to support development of bio-based products and processes, with a track record of enabling our customers to achieve commercialisation. Since opening in 2012, the centre has completed more than 1200 projects with over 650 partners from across the world, ranging from academia to small businesses, to global corporations. This presentation explores case studies in biomanufacturing process development and scale-up and the opportunities for new biological systems and processes to change the way we manufacture essential chemicals and materials.

PL-08

Biomanufacturing for Tomorrow, Today and Yesterday

Prof. Dr. Muhammad Safwan Akram*
Teesside University, United Kingdom.

ABSTRACT

In the last decade, we have seen great advances in biomimetics, gene therapies, biologics, biosimilars and treatment of orphan diseases. Capturing these advancements in the lecture, I would like to point out three big gaps that we need to fill to make these products sustainable. The first gap is the cost of these products and what healthcare systems can afford to pay. The second gap is the amount of the production facilities (cGMP) material required for the clinical studies required to push through clinical studies. The third gap is the lack of a skilled workforce to manufacture these products. The answer to the first gap lies in coming with innovative strategies to bring the costs down from artificial intelligence to industry 4.0 and designing better process analytical tools. In this regard, my lab has been working on glycan analytical tools to reduce the number of glycoforms. For the second and third gap, I would like to put forth Teesside's vision on how we have to change our curricula and prepare the next generation of scientists and engineers. This would include examples of using digital twins for training and starting a host of focused CPD programmes. I would also like to give some examples where my lab has been working with a company named Quorn to make yummy sausages and another company Calysta, to come up with a carbon negative feed for fish aquaculture. The lecture would also look at the challenges of developing nations with regards to drug quality.

PL-09

Harnessing the Regulatory Potential of 5′ UTRs to Enhancing Gene Expression in Plants

Prof. Dr. Kasra Esfahani*
National Institute of Genetic Engineering and Biotechnology, Iran.

ABSTRACT

Achieving optimal recombinant protein production in transgenic plants requires precise designing of regulatory regions of transgene expression constructs. The 5′ untranslated region (5′ UTR) is situated

at the 5′ end of protein-coding genes that are transcribed into mRNA but not translated into protein and plays a major role in controlling translation initiation due to its regulatory elements. Their impact on protein translation has led to the development of vectors with specific 5′ UTR sequences to enhance plant expression. Several companies now offer vectors featuring diverse 5′ UTRs. Notably, the 5′ UTRs of plant Alcohol Dehydrogenase (ADH), such as those from Arabidopsis and tobacco for dicotyledons and rice for monocotyledons, and the Tobacco Mosaic Virus Omega (Ω) leader sequence have been employed to improve translation efficiency. Vectors incorporating the Omega and *Arabidopsis thaliana* ADH 5′ UTRs were engineered to enhance gene expression at the translational level and the efficacy of these 5′ UTRs in modulating reporter gene expression was evaluated in transgenic barley plants.

SL-01

Investigating Host Immunity to Sars-Cov-2 Variants through the Pandemic in the Context of Different Covid-19 Vaccinations Administered in Pakistan

Prof. Dr. Zahra Hasan*
Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi.

ABSTRACT

The COVID-19 pandemic has underscored the significance of research in epidemiological surveillance of infectious diseases. SARS-CoV-2 evolved from the Wuhan strain (October 2019) into L, V and G clade strains, which later evolved into variants of concern (VOC) termed alpha, beta, gamma, delta and omicron variants, respectively. Vaccinations introduced in 2021 were mostly the inactivated virus types followed by one-dose vector vaccines and mRNA formulations. There was little information regarding the effect of these vaccines in the local population. We used a combined pathogen and host approach to investigate SARS-CoV-2 genomic diversity in the context of host immunity measured through molecular, microbiological, and immunological tools. Host responses to infection were studied through humoral immunity, antibody levels and neutralizing activity against viruses. RNA transcriptional profiles of individuals infected with SARS-CoV-2 variants were investigated. We studied COVID-19 vaccine responses in the population, following up individuals who received inactivated, one-dose vector and mRNA vaccinations. We found type I Interferon responses were key for early protection against COVID-19 and consistent with asymptomatic diseases. Compared with wild-type strains, VOC caused increased inflammatory responses and a down regulation of host heme synthesis pathways. In the context of vaccinations, SARS-CoV-2 Spike seropositivity was comparable between individuals vaccinated with different vaccine types, however, the magnitude of antibody responses was significantly greater after mRNA vaccinations. Host responses were reduced in those aged greater than 50 years. Virus neutralizing activity induced by COVID-19 vaccinations from 2021 was limited against new variants, although Spike seropositivity remained high. Our studies allow us to interrogate the drivers of immunity and factors associated with COVID-19 severity in a high infectious burden disease setting such as Pakistan. They also highlight the importance of research capacity building in an LMIC country.

SL-02

Designing Successful Serodiagnosis for TB Based on the Selected Antigens

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ABSTRACT

Tuberculosis (TB), caused by the intracellular pathogen *Mycobacterium tuberculosis* (*Mtb*), remains a leading cause of death from a single infectious agent. According to WHO Global *Tuberculosis Report 2024,* 8.2 million new TB cases were reported in 2023, up from 7.5 million in 2022, and an estimated 1·25 million deaths from TB in 2023. Pakistan continues to remain 5th highest contributor to the global TB burden. For effective TB control, the availability of a rapid, economical, and reliable diagnosis, particularly for the low-income and high-burden populations, is essential. Of the various diagnostic methods available, hardly any of these meet all the requirements. Serodiagnosis based on the detection of antibodies produced against the *Mtb* antigens has the potential, but due to the level of sensitivity falling short of the acceptable value, its application remained questionable. However, we propose that as the infection takes place the process of generation of antibodies against one or the other *Mtb* antigen shall commence. Thus, antigenic molecules having the capability of detecting the antibodies produced at different stages of the infection and the disease can enhance sensitivity and thus reliability of serodiagnosis to an acceptable level. We have designed and produced fusion molecules based on b-cell specific epitopes from multiple *Mtb* antigens specific to the latent, early or advanced stages of TB. Validation data for some of these fusions is highly promising, leading us to initiate pilot scale field study.

Keywords: *Tuberculosis, Serodiagnosis, Antigens, Antibodies.*

SL-03

Understanding the Epigenetic Impact of Hepatitis C Virus: Insights into Insulin Resistance as an Emerging Threat

Dr. Shazia Rafique*
CEMB, University of the Punjab, Lahore.

ABSTRACT

Hepatitis C virus is a significant public health concern effecting 10 million individuals in Pakistan with a viremia rate of 4.3%. Beyond its primary impact on liver function, it plays a key pathological factor for inducing insulin resistance. Such HCV induced insulin resistance is linked with type II diabetes, extrahepatic manifestations and the development of hepatocellular carcinoma. This study highlighted the significant involvement of epigenetic changes, especially DNA methylation dysregulation, in the pathogenesis of insulin resistance and its comorbidities. This study not only depicts HCV's role in metabolic dysfunction but also provides theoretical reference with regards to the impact of emerging pathogens on the epigenetic distribution of chronic liver diseases.

To investigate this relation, we applied EZ DNA Methylation-Gold™ Kit for probing Peripheral Blood Mononuclear Cells of HCV infected and non-infected individuals. Bisulfite treated DNA was

sequenced using the Illumina platforms. The significant differentially methylated probes (DMPs) and differentially methylated regions (DMRs) were determined using the Fisher's exact test with False Discovery Rate correction analysis. Gene Ontology enrichment analysis of 772 DMRs and 18,793 DMPs was performed using bioinformatics resources to counteract for gene length density. Using this criterion 12945 hypermethylated DMRs were screened, exhibiting the methylation difference of at least 0.1 and p value < 0.05. Additional pathway analysis of these DMRs showed that they were significantly involved in type II diabetes related pathways including PI3K-AKT/IRS1 signaling pathway, oxidative phosphorylation, the Renin-angiotensin system, and general metabolism. These findings provide a potential avenue for treatment plans that seek to address the long-term effects of HCV infection and associated metabolic diseases.

Keywords: *HCV, Insulin resistance, DNA methylation, Type-2 diabetes.*

SL-04

Determination of Resilience of a Panel of Broadly Neutralising MABs To Emerging Variants of Sars-Cov-2 Generated Using Reverse Genetics

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ABSTRACT

SARS-CoV-2 continues to evolve, and its emerging variants might escape the immune responses generated by existing vaccines and therapeutic mAbs. Accordingly, rapid analysis of their possible neutralisation phenotype is essential, and can be facilitated by reverse genetics systems to regenerate viruses with variant-specific substitutions. Here, we efficiently generate a panel of recent variants of SARS-CoV-2 (Omicron XBB.1.16, EG.5.1, BA.2.86 and JN.1) using a substantially optimized Circular Polymerase Extension Reaction (CPER) reverse genetics system. Neutralisation potency was analysed for mAbs targeting different regions of spike protein. mAbs P4-J15, C68.61, S2X259 and IY-2A IgG were able to neutralise all recent viruses. However, S309, which was previously used to treat infection and targets the outer face of RBD showed ~75-fold reduction in potency versus JN.1. Moreover, C68.59, which targets the SD1 region of the CTD, was unable to neutralise either BA.2.86

or JN1, which share the E554K substitution in SD1. CPER reverse genetics system and microneutralisation assays can be adopted as effective tools to evaluate the efficacy of therapeutic mAbs against emerging variants in a time-responsive manner.

Keywords: *Reverse genetics, CPER, Immune escape, IY-2A, P4-J15, C68.59, C68.61, BA.2.86, JN.1*

IL:01

Transforming Agriculture: Next-Generation Crop Resistance and Genome Editing Breakthroughs at CEMB

Abdul Qayyum Rao*, *Allah Bakhsh, Naila Shahid, Ayesha Latif, Saira Azam, Aneela Yasmeen, Tahir Rehman Samiullah, Ayesha Imran, Ujala Nasir, Sara Ajmal, Sahar Sadaqat, Muhammad Awais, Muhammad Saad Bhutta, Huda Mir, Sehar Zulfiqar, Sidra Qayyum, Noreen Iftikhar, Narmeen Tariq Zaman, Pashma Nawaz, Sania Naeem, Abdul Qadeer, Sana Fatima, Manahil Azhar, Rameen Ashraf Ali, Tasneem Nawaz, Munim Farooq, Muhammad Tariq, Muhammad Usman Arif and Farah Naz.*

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ABSTRACT

The Center of Excellence for Molecular Biology (CEMB) has led the development of insect and herbicide-resistant crops, securing commercial approval for their widespread cultivation across Pakistan. In response to the growing challenge of resistance buildup in insect pests, CEMB has advanced toxin levels through next-generation Bt technology and employed fusion gene approaches alongside cutting-edge genome editing techniques to create multi-herbicide-resistant cotton varieties. Moreover, the application of state-of-the-art genome editing tools such as CRISPR-Cas9 has proven essential in improving key crop traits. These advancements include the development of cotton varieties resistant to Cotton Leaf Curl Virus (CLCuV), submergence, salt, drought, and heat tolerance, as well as the creation of white-colored cotton. Additionally, CEMB has enhanced potato quality by extending shelf life, increasing vitamin A content, and improving resistance to fungal diseases, Potato Virus X (PVX), and Potato Virus Y (PVY).

Among the notable genome-editing achievements, CEMB successfully knocked out CLCuV-related DNA-A and Beta satellite components in cotton, and improved potato shelf life through VInv gene knockout. The knockout efficiency reached 72% for DNA-A and 90% for the Beta satellite, leading to a marked reduction in viral titers and 90% mortality in whitefly vectors. Structural validation using AlphaFold2 confirmed these successful edits at the protein level. Additionally, genetic analysis of edited potato lines revealed a significant reduction (90-99-fold) in VInv gene expression, alongside a fivefold decrease in reducing sugars in transgenic potato varieties. These results highlight the powerful potential of genome editing to combat plant viruses, enhance crop resilience, and address pressing agricultural challenges such as abiotic stress tolerance, paving the way for sustainable crop improvement in the future.

Keywords: *Crop Resistance, genome-editing, herbicide-resistant, Cotton Leaf Curl Virus.*

IL:02

Characterization of Metabolites of *Pseudomonas Chlororaphis* and Assessment of their Potential as Biocontrol Agent

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ABSTRACT

Fluorescent pseudomonad-based biofungicides have attracted considerable attention as effective biological control agents and biofertilizers. In this study, nine *Pseudomonas* isolates, PB-St2 (sugarcane), GS-1, GS-3, GS-4, GS-6, GS-7, FS-2 (cactus), ARS-38 (cotton), RP-4 (para grass) were evaluated for their antifungal potential and subjected to comprehensive metabolomic analysis. All isolates exhibited significant inhibition of fungal phytopathogens including *Fusarium* spp., *Alternaria* sp., *Rhizopus* sp., *Aspergillus* spp., and *Colletotrichum falcatum*. Based on biochemical tests and 16S rRNA gene sequences, PB-St2, GS-1, GS-3, GS-4, GS-6, GS-7, FS-2, and ARS-38 were identified as strains of *P. chlororaphis* subsp. *aurantiaca* and RP-4 as *P. chlororaphis* subsp. *chlororaphis*. All pseudomonads were characterized for the *in vitro* production of secondary metabolites in LB, DMB, and King's B media, and for the genes responsible for the production of antagonistic metabolites. ESI-LC-MS/MS data demonstrated that four phenazine derivatives, phenazine-1-carboxylic acid (PCA), 2-hydroxyphenazine-1-carboxylic acid (2-OH-Phz-1-COOH), phenazine1,6-dicarboxylic acid (Phz-1,6-di-COOH), and 2-hydroxyphenazine (2-OH-Phz), were produced by all strains in all three media. However, 2,8-dihydroxyphenazine, 6-methylphenazine-1-carboxylic acid, pyrrolnitrin, and the ortho-dialkyl-aromatic acids, were variably produced by the *P. aurantiaca* and *P. chlororaphis* strains. Additionally, all strains produced 2-acetamidophenol, pyochelin, lahorenoic acids A-C, and diketopiperazine derivatives in variable quantities in all three media. Differential levels of quorum-sensing signal molecules, including PQS, 2-octyl-3-hydroxy-4(1H)-quinolone, and hexahydro-quinoxaline-1,4-dioxide, were also noted in all strains. Furthermore, seven rhamnolipids including Rha-C_{10}–C_8, Rha-C_{10}–C_{10}, Rha-C_{10}–C_{12}/Rha-C_{12}–C_{10}, Rha-Rha-C_{10}–C_{10}, Rha-C_{10}-C_{12db}, Rha-Rha-C_{10}–C_{12}/Rha-Rha-C_{12}–C_{10}, Rha-Rha-C_{10}-C_{12db} were also detected in *Pseudomonas* sp. strains. Among acyl-homoserine lactones (AHLs), C_6-HSL, 3-OH- C_6-HSL, 3-OH- C_8-HSL, 3-oxo-C_6-HSL, 3-OH-C_{10}-HSL, 3-OH-C_{12}-HSL, 3-oxo-C_{12}-HSL, and 3-oxo-C_{12}-HSL were the predominant AHLs detected in *Pseudomonas* spp. strains. Moreover, all pseudomonads produced volatile HCN (0.95–6.68 µg/L) and the phytohormone IAA (0.42–13.9 µM). A comprehensive genomic analysis revealed the presence

of biosynthetic gene clusters encoding these compounds, rendering them promising candidates for biofungicide applications.

IL-03

Genetic Landscape of Retinal Dystrophies in Pakistani Families

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ABSTRACT

Retinal dystrophies (RDs) are a group of inherited diseases which result in the loss of vision. RDs are further divided into different types based on the clinical presentation of the patients but major types include Leber Congenital Amaurosis (LCA), Retinitis Pigmentosa (RP), Cone-Rod Dystrophy (CRD and Macular Degeneration (MD). Despite great progress on the identification of RD genes, the underlying genetic defect are still unknown in large number of cases/families. We have a cohort of 200 Pakistani families with RDs and performed genetic analysis in these families to identify disease causing mutations. Majority of the families belonged to LCA, whereas the remaining families were placed in RP and CRD group. Genetic analysis of these families with genome wide genotyping, homozygosity mapping, exome sequencing and Sanger sequencing resulted in the identification of underlying gene and mutations. Genetic findings of this cohort will be presented in the talk. This study provides insight to the genetic diversity of inherited retinal disorders in the Pakistani population and reports the identification of novel and known mutations in families segregating RDs. Genetic screening of such families that belong to remote areas with less resources and health facilities will help in accurate diagnosis and family counselling for further disease management.

IL-04

Identification of Novel Mutations in MCPH1 Gene in a Pakistani Family with Microcephaly

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ABSTRACT

Background: Primary microcephaly (MCPH) is a congenital, static, and non-progressive neurodevelopmental disorder associated with reduced head circumference (< 4 standard deviations). About 28 known genes are associated with MCPH. The study was carried out to probe molecular basis and genetic variants involved with MCPH in an affected Pakistani family to better understand the etiology and prevalence of the disorder.

Methods: The individuals of the ascertained Pakistani family presented primary microcephaly, along with intellectual disability, speech disorder, and motor delay. By ensuring ethical compliance and patient consent, blood samples were collected from affected individuals. DNA was extracted using the salting out method followed by whole-exome sequencing and Sanger sequencing to identify causative genetic variants or mutations. In silico studies were performed to predict the effect of mutations on the structure of target proteins.

Results: Two missense allelic variants (NM_024596.5: c.139G>C and NM_024596.5: c.211G>C) of MCPH1 gene were detected in a Pakistani family. In silico analysis was performed to evaluate the effect of the mutant protein. The mutation in genes affects the activities of proteins NM_024596: p. Val47Leu and NM_024596: p. Val71Leu respectively by disruption in protein structure. The mutations were predicted to have higher pathogenicity scores and have significantly influenced the prevalence of MCPH. We reported two novel genetic variants for the first time from the Pakistani population causing MCPH.

Conclusions: Mutations in the MCPH1 gene are one of the major causes of MCPH in the populations where consanguine marriages are common. The novel mutations identified in this study will help to understand the etiology of the disorder and the mechanisms of mutated proteins.

Keywords: *Primary microcephaly; MCPH; MCPH1; ASPM.*

IL: 05

Bioscience: Dry o Wet?

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ABSTRACT

My research career revolves around thermophilic microorganisms and the information hidden in their genomes. Genome sequences, no doubt, provide an enormous amount of information on the genes in a particular organism. Based on the assumption that genes with high similarity encode proteins of common function, the presence or absence of a specific metabolic pathway can be estimated in a particular organism. This approach, however, has a few limitations. Firstly, when an orthologue of an expected enzyme is not found, one must identify the gene through classical methods. Secondly, when multiple orthologues are present on the genome, one must carefully examine each gene product to distinguish the enzymatic functions.

During my career, I have encountered two types of researchers, *in silico*-philic and *in silico*-phobic. I shall try to explain, with examples from my research, that both types are becoming increasingly interdependent.

IL-06

Production and Characterization of Recombinant Enzymes for the Poultry Feed Industry

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ABSTRACT

Poultry sector is one of the major established industries of Pakistan that is committed to provide valuable meat to our community. Phytases, cellulases, xylanases and proteases are the main enzymes being added in the poultry feed. The addition of these enzymes is important because their addition in feed put a positive impact on the growth of poultry bird. Phytases are responsible for the availability of free phosphorus while xylanases and cellulases are responsible for the availability of monomeric absorbable sugars for the growth of birds whereas proteases also involve for the improvement of digestion of proteins. In the absence of these enzymes the phytate, cellulose and xylan are not being digested by the poultry bird and these components of feed simply pass through the digestive track and are removed from the body with manure and contribute in environmental pollution.

In the current study the phytase, cellulase and xylanase genes from hyper-thermophilic bacterium were amplified by using the PCR and ligated into the cloning vector pTZ57R/T. These vectors were transferred in the *E.coli* DH5α cells. The expression of phytase, cellulase and xylanase genes were analyzed in *E.coli* BL21CodonPlus cells with the help of pET expression system. Recombinant proteins were purified through different chromatographic techniques, and their molecular masses were determined through SDS-PAGE. Recombinant proteins were characterized. The locally produced recombinant enzymes were utilized for the supplementation of poultry feed to examine their effect on the growth of poultry birds. The supplementation of poultry feed with locally produced enzymes showed a significant growth enhancing effect on poultry birds and improved the feed uptake and feed conversion ratio.

***Keywords:** Poultry enzymes, Recombinant proteins, hyper-thermophilic bacterium.*

IL-07

Biotransformation of Agro-Industrial Wastes to Enzymes through Fermentation.

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ABSTRACT

Currently about 50-60% of waste is being generated from food and agro-industries which is not being properly used to produce value-added products. If this waste is not properly utilized, this ultimately leads to pollution problems and affects human and animal health. These wastes are rich in all nutrients required for the growth of microorganisms. These wastes could be converted to valuable products through microbial fermentation. Submerged fermentation and Solid-state fermentation are being widely used for the conversion of waste into valuable products such as animal feed and other valuable chemicals in the form of enzymes, biofuels and biopolymers. Microbes have the capability of transforming these wastes into valuable chemicals which leads to circular economy.

IL-08

Biosynthesis and Immobilization of Lignocellulosic Byproducts Derived Protease as a Robust Biocatalytic System for Various Industrial Applications

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ABSTRACT

In this study, different strains of *Aspergillus* were investigated for their ability to produce protease using a range of agricultural waste materials as substrates. *Aspergillus viridi* was the most productive strain, producing 83.0 U/mL of protease when cultivated in casein broth medium. The addition of 3% banana peels to PDB medium resulted in the highest enzyme production, reaching 172.6 U/mL. Optimizing the substrate-to-water ratio (1:3), incubation duration (72 hours), inoculum size (2 mL), pH (8), and temperature (35ºC) further increased protease production from 172.6 U/mL to 178.1, 180.2, 182.9, 188.7, and 193.1 U/mL, respectively. *A. viridi* utilized agricultural waste as its sole substrate and produced 202.8 U/mL under optimal conditions. Following partial purification using ammonium sulfate precipitation, the enzyme was further purified using chromatographic methods. With 80% ammonium sulfate precipitation, a purification fold of 1.37 and a specific activity of 136.9 U/mg were obtained. Subsequent chromatographic purification increased the specific activity from 136.9 U/mg to 308.5 U/mg and the purification fold from 1.37 to 3.09. SDS-PAGE analysis determined the molecular weight of the protease to be 45 kDa. Characterization studies revealed that the isolated protease exhibited maximum activity at pH 8 and 40ºC. The enzyme displayed a *Vmax* of 35.0 U/mL and a *Km* value of 38.2 mg/mL. The highest enzyme activity, 120.2%, was achieved when using 1% of a locally available detergent, Express Power. Additionally, the protease effectively removed the gelatin layer

from waste X-ray films within 48 hours. Furthermore, at alkaline pH 8, the enzyme degraded protein in soybean meals from 100% to 26.6%. The protease was immobilized using alginate beads. The highest immobilization yield of 74.4 U/mL was achieved with 3% sodium alginate, 4% calcium chloride, and 3 mL of protease enzyme. Characterization studies indicated that immobilization shifted the optimal pH from 8 to 9 and increased the optimal temperature from 40ºC to 45ºC. Kinetic analysis showed that, compared to free protease (*Km* = 30.1 mg/mL, *Vmax* = 37.0 U/mL), immobilized protease exhibited a lower *Km* of 30.1 mg/mL and a higher *Vmax* of 35.0 U/mL. When tested with 1% Express Power detergent, immobilized protease displayed maximum activity of 154.6%, compared to 120.2% for free protease. At pH 9, immobilized protease removed the gelatin layer from waste X-ray films in less than 24 hours. Additionally, it degraded protein in soybean meals from 100% to 13% at pH 9, whereas free protease reduced it only to 26% at pH 8. Stability studies showed that free protease retained 50% relative activity for up to 8 days, after which a sudden decline was observed. In contrast, immobilized protease maintained over 90% of the relative activity for up to 5 days when stored at 4ºC. Ultimately, these findings suggest that protease derived from fungi using agricultural waste holds significant potential for industrial applications.

IL-09

Engineering of Insulin Derivatives

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ABSTRACT

A groundbreaking discovery in medicine in the early 1920s was made by Banting and Best, along with Macleod and Collip, by identifying insulin, the first protein hormone. Isolated from the Islets of Langerhans, insulin became a beacon of hope for diabetics. It was also the first protein to have its primary structure fully determined, crystallized, and chemically synthesized. A major challenge in insulin synthesis is the correct formation of disulfide bridges between its polypeptide chains. The discovery of preproinsulin and proinsulin by Steiner provided critical insights into insulin biosynthesis. Preproinsulin, the initial product of insulin gene expression, undergoes signal peptide cleavage to form proinsulin, which consists of three domains: the A and B chains linked via the C-peptide. In the Golgi apparatus, proinsulin is further processed to remove the C-peptide, yielding the mature insulin molecule with two interchain and one intrachain disulfide bonds. Upon glucose elevation, insulin is released into the bloodstream to regulate blood sugar levels. Understanding insulin synthesis and processing has paved the way for recombinant insulin derivatives with tailored pharmacokinetics.

Rapid-acting analogs exhibit faster onset and shorter duration, while long-acting analogs provide stable and prolonged effects. This study explores the properties of insulin variants produced at SBS, Lahore. The process involves designing DNA constructs encoding insulin derivatives, expressing them heterologously in *Escherichia coli*, and subjecting the expressed proteins to proper refolding, purification process, chemical modifications and finally converting into a bioactive hormone using tryptic-cum-carboxypeptidaseB treatment. The resulting insulin derivatives demonstrate biological activity comparable to commercially available recombinant human insulin. The future of insulin variants holds great promise, with potential advancements that could revolutionize diabetes management and continue to provide life-saving treatment for millions worldwide.

IL-10

Development of Intranasal Formulation of Z-Acid as Neuroprotective Agent and Temporin Sha Analogs as New Anticancer Peptides

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ABSTRACT

This study presents the discovery and development of Z-Acid, a novel antiepileptic and neuroprotective compound derived from a natural product isoxyiltones which was previously identified as active principle of plant *Delphinium denudatum*. This plant is traditionally used in fold medicine for its anti-convulsant properties. Pre-clinical research has demonstrated that Z-Acid effectively inhibits seizures and reduces ischemic brain injury in animal models. The compound was found to be highly potent, with superior bioavailability when administered intranasally compared to traditional methods. Z-Acid displayed excellent safety profiles, showing no signs of mutagenicity, cytotoxicity, or hemolysis. It proved significantly more efficacious then the existing anti-convulsant drugs in pre-clinical trials. Supported by extensive *vitro* and *in vivo* testing, the Z-Acid nasal formulation offers a promising and cost-effective treatment option for patients suffering from epilepsy and stroke. Currently, the work is progressing towards Phase-1 clinical trials, with ethical approvals secured and patent protection in multiple regions. This research marks a significant step forward in developing the effective, safe and accessible treatment option for central nervous system disorders.

Moreover, various linear, cyclic, dendrimeric, and drug (levofloxacin) conjugated analogues of the temporin SHa peptide, isolated from the skin of *Pelophylax saharicus* frog, were synthesized and their anticancer and antimicrobial activities were evaluated. The biological studies of SHa peptide analogs showed the great potential of these peptides to serve as powerful therapeutic agents in the ongoing quest for new drug discoveries.

IL-11

Structural, Functional and Immunological Characterization of an Outer Membrane Protein, FrpBDr.

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ABSTRACT

Membrane proteins play a major role in the transport of solutes and macromolecules across cell membranes which is one of the vital cellular processes. Import of solutes like metal ions inside the cell from the surrounding environment through various uptake systems is essential for cell survival. The importance of iron uptake to pathogenic Neisseria is well established, with multiple uptake systems which can extract iron from transferrin, lactoferrin and heme. FrpB is an outer membrane transporter from Neisseria meningitidis, the causative agent of meningococcal meningitis, involved in the transport of iron into the periplasm. It is a member of the well-studied TonB-dependent transporter (TBDT) family. However Much less attention has been paid to the role of TBDTs as antigens. FrpB is subject to a high degree of antigenic variation, principally through a region of hypervariable sequence exposed at the cell surface. From the crystal structures of two FrpB antigenic variants, we identify a bound ferric ion within the structure which induces structural changes on binding which are consistent with it being the transported substrate. EPR spectra of the bound Fe3+ ion confirmed that its chemical environment was consistent with that observed in the crystal structure. Fe3+ binding was reduced or abolished on mutation of the Fe3+-chelating residues. FrpB orthologs were identified in other Gram-negative bacteria which showed absolute conservation of the coordinating residues, suggesting the existence of a specific TBDT sub-family dedicated to the transport of Fe3+. The region of antigenic hypervariability lies in a separate, external sub-domain, whose structure is conserved in both the F3-3 and F5-1 variants, despite their sequence divergence. We conclude that the antigenic sub-domain has arisen separately because of immune selection pressure to distract the immune response from the primary transport function.

IL-12

Virulence and Antibiotic Resistance Modulation by Paerucumarin in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogenic bacillus that is notorious for hospital-born nosocomial infections. It causes severe health issues in humans by causing infections in various body

parts through an arsenal of virulence factors. Bacterium selectively releases exotoxins, elastases, proteases, exopolysaccharides, pyocyanins, alginates, and sidrophores after sensing the surrounding environment via its unique quorum sensing system. In burn mouse and diabetic mouse models, it forms antibiotic-resistant bacterial communities called biofilms. Paerucumarin is a comparatively novel metabolite produced by the sequential action of proteins encoded by *pvcABCD* operon, which modulates the quorum sensing system of *P. aeruginosa* under various environmental conditions. This metabolite enhances biofilm formation in culture media by modulating the expression of fimbrial chaperone-usher pathway encoding genes. The precursor of paerucumarin, i.e. isonitrile functionalized tyrosine, enhances rhamnolipid production, which promotes quorum sensing and swarming motility of *P. aeruginosa*. Moreover, the deletion of *pvcB* gene shuts down mexEF-oprN efflux pump, thus making bacteria more sensitive to antibiotic resistance. All these findings make paerucumarin a very interesting metabolite to be studied with reference to its genetic control and therapeutic potential.

IL-13

Next-Generation Sequencing is a Compulsion and not an Option in Molecular Diagnostics

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ABSTRACT

Data analysis of next-generation sequencing for causative variants identification in single gene disorders has been exceptionally increased both in molecular diagnostics and research setups. In the current genomic era, next-generation sequencing has been widely adopted as a first line diagnostic tool. However, precise data analysis pipelines are required at each clinical setup for efficient and accurate variant prioritization. In this work, we have programmed our own data analysis pipeline named as “VARDIGS”, an NGS data analysis pipeline, using Snakemake and open-source tools (FastQC, Picard, BWA, GATK, ANNOVAR, CONIFER). The script is based on bespoke which uses causative variants (missense/non-sense/small indels) prioritization in a WES data according to the ACMG criteria. The second application of the tools is “Exscope”, another Python tool, which can detect exonic deletions in a WES data. Furthermore, the gene interaction and pathways are analyzed with David bioinformatics, StringDB, Reactome, and KEGG databases. Analyzing our in-house WES sequencing data we have performed successful analysis of several samples, and the tool has successfully shortlisted/detected the causative variants. Particularly, in the sample EPB21-ABTX we detected the GHR isoform with missing exon 8 suggestive of Laron dwarfism phenotype. In the sample EPB21-BFYN we detected compound heterozygous mutations on exon 3 in the FLG gene along with

deleted exon 1. Furthermore, we detected two homozygous variants in distinct gene MAG and PRX classified as VUS and Pathogenic respectively in the sample EPB21-BONU. The VARDIGS pipeline's integration within our lab enhances rare disease genetics and genomic research by employing new bioinformatics tools for scalable, efficient NGS data processing. It simplifies processing, improves consistency, and speeds up the discovery of pathogenic mutations, hence promoting early diagnosis and precision treatment. This research emphasizes VARDIGS as a critical connection between sophisticated genetic research and clinical diagnostics, hence supporting future personalized medicine breakthroughs.

IL-14

London-Pakistan Parkinson Project

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ABSTRACT

Parkinson's disease (PD) is the second most prevalent neurological disorder globally. In Pakistan, a country with a population of approximately 240 million, the genetic and environmental factors contributing to PD's high prevalence remain poorly understood. A lack of awareness and the misconception that PD is solely associated with aging often result in patients seeking treatment at later stages of the disease. This study aims to investigate the genetic causes of PD in the Pakistani population through a collaborative effort involving two distinct sites in Lahore and Islamabad. These locations have been selected to capture variability in socio-economic status and ethnic diversity. Our objective is to recruit 600 participants over the course of one year, including 400 PD patients and 200 age-matched controls without neurological disorders. Additionally, 90 cerebrospinal fluid (CSF) samples will be collected. The study will focus on measuring biomarkers of neuronal damage and related processes, such as NFL, GFAP, Aβ-40, Aβ-42, p-Tau 181, and p-Tau 217. We will also analyse alpha synuclein in blood and CSF samples using the alpha-synuclein seed amplification assay (SAA) technique and/or measurement of total synuclein in extracellular vehicles (EVs).Participant data will be shared in compliance with the Global Parkinson's Genetics Program (GP2) data-sharing policies, ensuring confidentiality.This research will provide critical insights into the genetic factors of Parkinson's disease in the Pakistani population, contributing to the advancement of PD-related health research and the development of improved management strategies for the disease.

IL-15

Exploring Artificial Neural Networks and Nano Informatics for *Bacillus Cereus*-Based Synthesis of Cysteine-Conjugated Bimetallic Nanoparticles for Diesel Degradation

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ABSTRACT

The increasing environmental pollution caused by diesel fuel has prompted the development of innovative, sustainable methods for its degradation. One promising approach involves the use of Bacillus cereus, a versatile microorganism known for its metabolic capabilities, to synthesize bimetallic nanoparticles for environmental applications. In this study, we explore the potential of combining Artificial Neural Networks (ANN) and Nanoinformatics to optimize the synthesis of cysteine-conjugated bimetallic nanoparticles by Bacillus cereus for effective diesel degradation. The integration of ANN provides a data-driven approach to model and predict synthesis parameters, enhancing the efficiency and yield of the nanoparticles. Nanoinformatics, on the other hand, aids in the characterization and design of these nanoparticles at the molecular level, offering insights into their structural properties and their interaction with diesel contaminants. Through this interdisciplinary approach, we demonstrate the successful synthesis of highly effective nanoparticles with superior catalytic activity for the breakdown of diesel, thus contributing to a cleaner, more sustainable environment. This work opens new avenues for the application of biotechnology and nanotechnology in environmental remediation and provides a novel perspective on utilizing microorganisms for advanced material synthesis in the context of environmental cleanup.

Keywords: *Bacillus cereus, Artificial Neural Networks, Nanoinformatics, Cysteine-Conjugated Bimetallic Nanoparticles, Diesel Degradation.*

IL-16

Redefining Molecular Boundaries: The AI Renaissance in Protein Structure and Drug Development

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ABSTRACT

Artificial intelligence (AI) has brought about a paradigm shift in biochemistry, medicine and pharmaceutical research by increasing the efficiency, accuracy and reliability of protein structure modeling and drug discovery. AI-driven approaches can be combined with the existing protein modeling methods, advanced molecular dynamics (MD) simulations, and predictions of protein-ligand interactions. This merger transforms our understanding of molecular interactions and accelerates the identification of potential therapeutic compounds. This transformation involves the application of

machine learning and deep learning methods in protein structure prediction and in silico drug design. AI helps researchers to forecast protein-ligand interactions accurately, assess toxicity, and conduct pharmacokinetic studies in a shorter time and at reduced costs compared to traditional approaches. MD simulations further strengthen these insights by sorting detailed snapshots of molecular behavior for a deeper exploration of conformational dynamics and stability, which is necessary for effective drug targeting. This talk will illustrate how these computational tools integrate with AI to optimize the discovery pipeline. By getting help from real-world cases, I will show the successful application of AI in drug development, starting from early virtual screening to lead optimization. The future challenges, like obtaining high-quality but reliable data, mitigating algorithmic biases, and enhancing the interpretability of AI-generated models, are becoming debatable. By combining the theoretical frameworks and practical implementation, the aim is to encourage researchers to make possible ethical use of AI tools for protein modeling and MD simulations in their research. It also envisions a future where these complementary technologies are fully embedded within the biochemistry labs, molecular biology processes, and drug development lifecycles, revolutionizing how we conceptualize, design, and deliver next-generation therapies.

IL-17

Statistical Mechanics Approaches to Develop Combinatorial Cancer Therapeutics

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ABSTRACT

Multi-scale models, integrating biomolecular data from genetic, transcriptional, and translational levels, coupled with extracellular microenvironments, decode complex mechanisms in diseases like cancer. To investigate the emergent properties and clinical translation of such cancer models, we present the Theatre for in silico Systems Oncology (TISON, https://tison.lums.edu.pk), a next-generation web-based multi-scale modeling and simulation platform for in silico systems oncology. TISON offers a zero-code environment for multi-scale model development, seamlessly coupling information from biomolecular networks, microenvironments, cell decision circuits, silico cell lines, and organoid geometries. Its simulation engine and data analysis features compute spatio-temporal evolution of *in-silico* models. TISON integrates patient-specific gene expression data for personalized therapeutics. Several case studies, including multi-scale colorectal cancer, validate TISON's capabilities, supporting patient-specific network modeling, simulations, and data analytics toward personalized treatments.

IL-18

Artificial Photosynthesis and Artificial Intelligence (AI): Efficient Conversion of Solar Energy and Water into Fuels

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ABSTRACT

With the advent of innovative molecular science, chemical technology and artificial intelligence (AI), nanoscale materials can be engineered and programmed to perform specified function at macro level applications. The revolution in chemical science, nanomaterials, catalysis, and electrochemical processes for Water Splitting has a lead now for solar and chemical energy conversion. These systems can be implemented as surface immobilization along with thin-films for catalytic processes, sensing applications and for energy conversion schemes. We have invented, discovered and developed specialized methods, and exploited various thin-film nanoscale materials for catalytic water splitting, CO2 reduction, and recently for electrochemical sensing, biomass catalysis and solar energy conversion. Now we implement and developing new methods for making advanced electrofunctional nanomaterials and nanoclusters derived from thin-films molecular assemblies, inorganic nanomaterials and metal-oxides displaying great potential to be used in high performance water splitting catalysis and for chemical energy conversion and storage schemes. In this discussion we also highlight the challenges in chemical energy conversion and the possible way forward.

IL-19

Regulating Plant Metabolism for Stress Resilience

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ABSTRACT

Water scarcity is a serious agricultural problem causing significant losses to crop yield and product quality. The development of technologies to mitigate the damage caused by drought stress is essential for ensuring a sustainable food supply for the increasing global population. We herein report that the exogenous application of ethanol, an inexpensive and environmentally friendly chemical, significantly enhances drought tolerance in Arabidopsis thaliana, rice, and wheat. The transcriptomic analyses of ethanol-treated plants revealed the upregulation of genes related to sucrose and starch metabolism, phenylpropanoids, and glucosinolate biosynthesis, while metabolomic analysis showed an increased

accumulation of sugars, glucosinolates, and drought-tolerance-related amino acids. The phenotyping analysis indicated that drought-induced water loss was delayed in the ethanol-treated plants. Furthermore, ethanol treatment induced stomatal closure, resulting in decreased transpiration rate and increased leaf water contents under drought stress conditions. The ethanol treatment did not enhance drought tolerance in the mutant of ABI1, a negative regulator of abscisic acid (ABA) signaling in Arabidopsis, indicating that ABA signaling contributes to ethanol-mediated drought tolerance. The nuclear magnetic resonance analysis using 13C-labeled ethanol indicated that gluconeogenesis is involved in the accumulation of sugars. The ethanol treatment did not enhance the drought tolerance in the aldehyde dehydrogenase (aldh) triple mutant (aldh2b4/aldh2b7/aldh2c4). These results show that ABA signaling, and acetic acid biosynthesis are involved in ethanol-mediated drought tolerance and that chemical priming through ethanol application regulates sugar accumulation and gluconeogenesis, leading to enhanced drought tolerance and sustained plant growth. These findings highlight that stress response could be regulated through GM as well as non-GM technologies.

IL-20

Unravelling RXLR Effector Proteins in Transgenic Potato: A Key to Enhancing Protection Against Late Blight Disease Pathogenesis

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ABSTRACT

Potato plants defend themselves with toxic compounds called steroidal glycoalkaloids (SGAs). The potato pathogen Phytophthora infestans counters this defense by producing a protein, PITG_19230, which breaks down SGAs. This protein, a rhamnosidase, removes sugar molecules from SGAs, reducing their toxicity. We have showed that potato plants expressing PITG_19230 were more susceptible to P. infestans infection, exhibited reduced SGA levels, and suffered more severe disease symptoms. Molecular analysis confirmed that PITG_19230 strongly interacts with SGAs, particularly α-chaconine. Thus, PITG_19230 is a virulence factor that helps *P. infestans* overcome potato defenses, potentially informing future potato breeding strategies.

Keywords: *RXLR effectors, Transgenic potato, Late blight, Phytophthora infestans.*

IL-21

Cell-based Cardiac Regeneration: Role of Wnt Pathway Modulators

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ABSTRACT

Reprogramming of mesenchymal stem cells (MSCs) into cardiac progenitor cells is a significant approach in regenerative medicine for treating cardiovascular disorders. Wnt/β-catenin, a highly conserved signaling pathway, is involved in determining cell fate. During heart development, Wnt signaling controls specification, proliferation and differentiation of cardiac cells. This study is aimed to investigate the role of Wnt/β-catenin signaling in cardiac lineage commitment of human umbilical cord mesenchymal stem cells (hUC-MSCs). hUC-MSCs were treated with Wnt pathway modulators and analyzed for Wnt pathway and cardiac lineage commitment at gene and protein levels. Significant upregulation of early and late cardiac markers, GATA4, Nkx2.5, cardiac myosin heavy chain (cMHC), α-actinin, cardiac troponin T (cTnT) and cardiac troponin I (cTnI) was observed in treated MSCs as compared to the untreated control. We also analyzed gene expression of key Wnt/β-catenin signaling molecules in cultures of treated and untreated hUCMSCs at 24 h, and days 3, 7 and 14. The pattern of mRNA gene expression showed that Wnt/ β-catenin signaling is regulated during cardiac lineage commitment of hUCMSCs in a time-dependent manner, with the pathway being activated early but inhibited later in cardiac development. The in-vivo study confirmed the induction of myocardial infarction and showed improvement in cardiac functional parameters and homing of cells, demonstrating the regenerative potential of the transplanted cells. In conclusion, this study demonstrated that treatment of hUC-MSCs with Wnt pathway modulators can be chemically induced to differentiate toward the cardiac lineage in vitro and in vivo. The cardiac differentiation potential of these modified stem cells can be clinically tested in future. However, further optimization is necessary to enhance the functionality of the fully differentiated cardiac tissue.

Keywords: *Mesenchymal stem cells, cellular differentiation, reprogramming, cardiac regeneration, Wnt pathway.*

IL-22

Stem Cells Priming Augments the Therapeutic Potency of Stem Cells for Wound Repair

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ABSTRACT

Background: Persistent hypoxia at the wound site in diabetic and burnt patients challenge the viability and therapeutic potency of transplanted stem cells. Hence current studies aimed to investigate the priming effect of Curcumin and 2-deoxy-D-ribose (2dDR) in improving their ability to tolerate hypoxic stress *invitro* with the subsequent examination of 2dDR primed ASCs for healing of excisional wounds in streptozotocin (STZ)-induced diabetic rats' model *invivo*.

Methods: For this, impact of 2dDR priming on ASCs against cobalt chloride mimicked hypoxic stress was evaluated through viability by MTT assay, senescence by β-galactosidase staining, migration by scratch healing assays, ROS measurement, hypoxia-inducible factor-1α (HIF-1α) expression and regulation of AKT and ERK pathways. Similarly, curcumin primed stem cells were investigated invitro for their ability to counter hyperglycemia stress *invitro* by viability, cytotoxicity, senescence, and scratch wound healing assays. Further, invivo investigation was performed in both studies by implanting 2dDR primed ASCs and Curmin primed ASCs in wounds in diabetic rats. Post-transplantation, wounds were photographed at intervals to evaluate percent wound closure. Healed wound biopsies were excised for histological evaluation by hematoxylin and eosin, masons' trichrome staining's and gene expression analysis of wound healing markers by real time-PCR.

Results: 2dDR-ASCs counteracted the hypoxic stress significantly better than unprimed ASCs and maintained their viability and migration potential. 2dDR priming led to senescence reduction, deterrence in ROS accumulation and stabilized HIF-1α expression, thus exerted its protective effects via synergistic activation of AKT and ERK pathways. *In vivo* results showed that wounds treated with 2dDR-ASCs were earlier healed with restored architecture (thick epidermis, evident granulation, marked collagen deposition) comparative to other groups. Also, mRNA expression level of key healing markers i.e., epidermal (CK-1, CK-10), dermal (Vimentin, TGF-β1, COL1α1) and angiogenesis (HIF-1α, FGF-2, VEGF) markers was found upregulated in cells treated groups versus saline treatment but more prominent in 2dDR-ASCs injected group comparative to unprimed ASCs. Further, Curcumin primed ASCs resulted in faster wound closure, improved fibroblast proliferation,

increased neovascularization, marked reduction in inflammatory cells, and compact extracellular matrix with completely covered thick epithelium.

Conclusion: This study findings substantiate that 2dDR and curcumin priming of ASCs exert cytoprotective effects against hypoxia and hyperglycemia respectively and propose priming mediated stem cell-based therapies for management of diabetic wounds.

IL-23

Identification of New and Potent Inhibitors for DHFR as a Chemotherapeutic Agent Against Cancer Using Machine Learning-Based Virtual Screening and *In vitro* Assays

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ABSTRACT

Dihydrofolate reductase (DHFR) plays a crucial role in folate metabolism, driving essential redox reactions that are vital for protein and nucleotide biosynthesis. This makes DHFR an attractive target for drug development, particularly in the treatment of life-threatening diseases such as cancer. In this study, we employed a combination of in silico and in vitro approaches, including machine learning-based virtual screening of in-house and Zinc databases, followed by molecular docking and molecular dynamics simulations. Virtual screening yielded 161 hits from the in-house database and 373 hits from the Zinc database. After applying stringent drug-likeness criteria, 149 in-house hits and 373 Zinc hits underwent docking analysis to assess their binding interactions and stability in complex with DHFR. Top candidates, selected based on superior docking scores, strong binding interactions, and favorable drug-likeness properties as determined by ADMET and PAINS analysis, were subjected to molecular dynamics simulations to evaluate their dynamic stability, binding free energies, and binding affinities. Three compounds—qtme-12, qtme-14, and SBEH-40—were selected for further in vitro validation through biochemical assays. The results revealed that qtme-14 and qtme-12 exhibited comparable IC50 values of 14.80 ± 0.30 and 21.11 ± 0.36, with inhibitory activities of 89.60% and 87.40%, respectively. In contrast, SBEH-40 showed relatively weak activity, with an IC50 value of 26.15 ± 0.47. Interestingly, all three compounds are natural products derived from the aloe vera plant, suggesting they may possess a lower risk of side effects, as indicated by the ADMET analysis. These findings provide a comprehensive evaluation of potential DHFR inhibitors and may contribute to the development of novel chemotherapeutic agents for cancer treatment.

Keywords: *DHFR, Machine learning, Enzyme inhibition, Molecular docking, ADMET.*

IL-24

Dentification of NS2B-NS3 Protease Inhibitors for Therapeutic Application in ZIKV infection: a Pharmacophore-Based High-throughput Virtual Screening and MD Simulations Approaches

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ABSTRACT

The Zika virus (ZIKV) pandemic and its implication in congenital malformations and severe neurological disorders have created serious threats to global health. ZIKV is a mosquito-borne flavivirus that spreads rapidly and infects many people in a short period. Due to the lack of effective therapeutics, it has become of paramount urgency to discover effective drug molecules to counter viral infection. Various anti-ZIKV drug discovery efforts during the past several years have been unsuccessful in developing an effective cure. The NS2B-NS3 protein was reported as an attractive therapeutic target for inhibiting viral proliferation due to its central role in viral replication and the maturation of non-structural viral proteins. Therefore, the current in silico drug exploration aimed to identify the novel inhibitors of Zika NS2B-NS3 protease by implementing an e-pharmacophore-based high-throughput virtual screening. A 3D e-pharmacophore model was generated based on the five-featured (ADPRR) pharmacophore hypothesis. Subsequently, the predicted model is further subjected to the high-throughput virtual screening to reveal top hit molecules from the various small molecule databases. Initial hits were examined in terms of binding free energies and ADME properties to identify the candidate hit by exhibiting a favourable pharmacokinetic profile. Eventually, molecular dynamic (MD) simulation studies were conducted to evaluate the binding stability of the hit molecule inside the receptor cavity. The findings of the *in-silico* analysis provided affirmative evidence for three hit molecules with binding free energies of -64.28, -55.15, and -50.16 kcal/mol, indicating them as potent inhibitors of the Zika NS2B-NS3 protease. Hence, these molecules hold the promising potential to serve as prospective candidates for designing effective drugs against ZIKV and related viral infections.

Keywords: *ASINEX database; Zika virus; e-pharmacophore approach; high-throughput virtual screening; molecular dynamics simulation; prime MM-GBSA.*

IL-25

Exploring Synergistic Interactions Between Natural Bioactive Molecules and Arsenic Trioxide in Liver Cancer: A Novel Approach to Reduce Systemic Toxicity and Improve Therapeutic Outcomes of Rrsenic Trioxide

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ABSTRACT

Despite continuous therapeutic interventions, the prognosis of hepatocellular carcinoma (HCC) remains very poor. Thus, the quest for novel treatment strategies to improve therapeutic window of HCC therapy is paramount. Arsenic trioxide (ATO) is commonly used as the first-line treatment for acute promyelocytic leukemia (APL). Recent research has shown that ATO exhibits antitumor efficacy against multiple solid tumors however, it also causes serious systemic toxicity at high doses. The present study aimed to investigate the potential synergistic effects of combination of natural bioactive molecules and low dose ATO in HCC cells to reduce systemic toxicity and improve antitumor efficacy of ATO. Among various tested compounds, Isoliquiritigenin (ISL) exhibits promising synergistic anticancer effects. The data revealed that the combination of ISL and ATO synergistically inhibited HCC cell proliferation. The collective data demonstrates that synergistic anticancer effect of combined treatment of ISL+ATO was achieved via cooperative induction of mitochondrial apoptosis through ROS generation and inhibition of PI3K/Akt/mTOR pathway. In addition, ROS generation and suppression of PI3K/Akt/mTOR pathway were found to be two independent events in induction of apoptosis. Finally, we observed that combination treatment effectively suppressed tumor growth in nude mice xenograft model through induction of intrinsic apoptosis and inhibition of PI3K/Akt/mTOR pathway. In conclusion, the findings of this study suggest that both drugs work synergistically to exert anti-tumor effect in HCC, both *in vitro* and *in vivo* and could offer novel strategy for liver cancer treatment.

IL-26

Genomic Insights into Yak (*Bos grunniens*): The Keystone Species for Food Security and Sustainable Livelihoods in Northern Pakistan's Changing Climate

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ABSTRACT

Yak (*Bos grunniens*), a crucial species for high-altitude communities, are facing threats from climate change due to rising temperatures, shifting precipitation patterns, and degradation of alpine pastures. They also reduce quality forage availability, increase heat stress, disease outbreaks, and declining reproductive rates. The decline in yak population is a great threat to food security for mountain communities depending on yak products such as meat, milk, fiber and transport. To further increase

the capacity of yaks to endure, a series of comprehensive conservation strategies that encompass traditional knowledge with findings from science must be implemented, ranging from sustainable pasture management, genetic conservation, sustainable breeding, disease monitoring and community-based adaptation strategies. For this our research group at Virtual University of Pakistan has been actively working on series of different projects like genetic screening and conservation, sustainable breeding and adaptation strategies for more enhanced production of yak and its products. We have conducted genetic studies on *ANK1* (for improving meat quality), *IL2* (promote the development of T cells), *DGAT1* (involved in maintain meat quality), *PPARA* (involved in fat metabolism during energy stress), *PRKAA1* (activates pathway involved in maintaining energy balance), *HIF1A* (regulates metabolic process under hypoxia), *VEGFA* (stimulates angiogenesis to increase vascularization in response to hypoxia) genes and found some known as well as novel mutations. Along with that parasitic study has also been performed and found molecular prevalence of tick borne *Anaplasma marginale* and *Theileria ovis*. We have also developed collaboration from Livestock and Dairy Department, Gilgit-Baltistan, BZU, Multan and UVAS, Lahore. Our efforts are directed towards developing adaptive strategies to mitigate these impacts and ensure the resilience of yak-dependent communities. Protecting yak populations is not just a matter of biodiversity conservation; it is essential for maintaining the food systems, cultural identity, and economic stability of millions living in fragile mountain ecosystems.

Keywords: *Yak (Bos grunniens), Genomics, Food security, Global Climate Change, Pakistan*

IL-27

Novel Hit Discovery for Dengue Virus (DENV) RNA-Dependent RNA Polymerase (RdRp)

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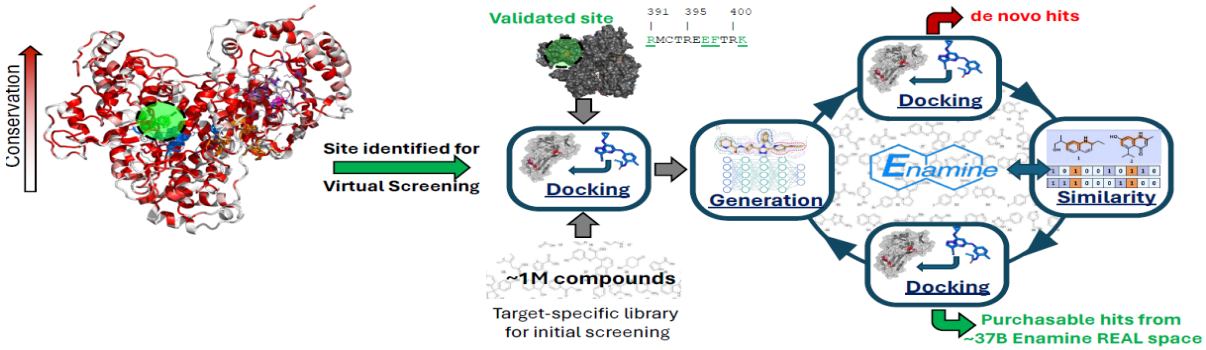
ABSTRACT

Dengue fever remains a significant global health challenge and affects millions of individuals globally. Considering the lack of antiviral treatment options for the disease, we have been interested in identifying novel hits capable of limiting viral multiplication. In this regard, we have been particularly interested in viral RNA-dependent RNA polymerase (RdRp). This enzyme catalyzes the synthesis of viral RNA from an RNA template and is a key player in the viral replication process. Inhibition of RdRp can disrupt viral replication and reduce the viral load and severity of the disease, and since RdRp is conserved across different DENV serotypes, the development of a RdRp inhibitor could lead to a broad-spectrum antiviral. Thus, we have adopted a multi-faceted approach involving numerous discovery campaigns to identify potential leads for the DENV RdRp. These campaigns include Virtual

screening, DNA encoded libraries, Fragment screening and Affinity selection mass spectrometry. This talk will present a brief overview of these approaches and the progress in identifying the novel hits so far.



OP-01

A Biochemical and Molecular Docking Insight Towards Bio-Protection of Rice Plants Against Brown Leaf Spot Diseases by *Bacillus Megaterium* Strain Z-06

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ABSTRACT

Plant growth promoting rhizospheric bacteria belonging to Bacillus genera can promote plant growth and manage biotic stresses which makes them an interesting tool for agriculture practices. Current study was performed to explore the potential of different strains of rhizospheric Bacillus bacteria to suppress brown leaf spot disease of tomato along with growth promotion. Bacteria were applied as seed priming by dipping them in aqueous suspension of bacterial strains overnight. After one month of emergence, rice plants were inoculated with the virulent strain of Bipolaris oryzae. Among them, B. megaterium strain Z-06 effectively suppressed brown leaf spot disease of rice. B. megaterium strain Z-06 reduced disease index up to 63.98% compared to disease control. Furthermore, Z-06 primed rice plants with induced defense responses. Z-06 increased the production of total phenolic compounds and activities of enzymes involved in phenylpropanoid pathway. Non-targeted metabolomic analysis was performed to elucidate the mechanisms behind disease suppression and plant growth promotion. The onset of pathogen significantly reduced the production of a range of primary and secondary metabolites including sugars, amino acids, fatty acids, sugar alcohols, polyols, and organic acids. Whereas the application of Z-06 increased production of number of metabolites in rice plants wither alone or in the presence of leaf spot pathogen. Lastly, Molecular docking analysis was performed to putatively screen biochemicals/elicitors secreted by Z-06 bacterium capable of docking with cytoplasmic kinase Broad-Spectrum Resistance 1 (BSR1) receptor of rice plants responsible for primmed defense responses. Nitrophenazine showed strong affinity with the rice BSR1 protein as shown by the molecular docking analysis. The findings of this study indicate that B. megaterium stain Z-06 is an effective biocontrol agent and has the potential to be used under a conventional agricultural system as a biocontrol and biostimulator agent.

Keywords: *Brown leaf spots, Bipolaris oryzae, B. megaterium, Induced Resistance, PGPR.*

OP-02

Exploring the Genetic Basis of the Stay-green Trait in Bread Wheat: A GWAS Approach

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ABSTRACT

Global climate extremes, coupled with the decline in arable land quality and quantity, are expected to significantly impact global cereal production. To meet the food demands of a projected 9 billion people by 2050, crop yields must increase by 70%. To address yield losses caused by climate extremes and boost production, sustainable strategies for improving yields are essential. The stay-green trait, which enhances grain mass by maintaining chlorophyll content, photosynthetic capacity, and carbon supply during the final growth stages under stress, serves as a vital adaptive mechanism. However, the genetic basis of this trait in wheat remains poorly understood. This study investigates the genetic underpinnings of the stay-green trait using a diverse set of wheat germplasm, including landraces, green revolution, post-green revolution, and elite cultivars, through GWAS methods (GLM, MLM, and FarmCPU). A range of morpho-physiological traits, such as chlorophyll content, chlorophyll fluorescence, NDVI, plant height, tiller number, spike length, spikelets per spike, thousand kernel weight, grain yield, and biological yield, were evaluated over field trials for five years. Analysis revealed significant genotype differences and positive correlations between stay-green indices and grain yield. The GWAS identified 83 significant marker-trait associations across 48 loci. Among these, 20 loci were further investigated for gene identification, revealing 342 high-confidence protein-coding genes, 36 of which were linked to stress tolerance, and chloroplast development. These findings offer valuable insights for future research aimed at identifying candidate genes controlling the stay-green trait in wheat.

Keywords: *Wheat; Stay-green; GWAS.*

OP-03

CRISPR/Cas9 Mediated Mutagenesis of VISCOSITY 1 Gene for Tomato Shelf-Life Enhancement

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ABSTRACT

Tomato (*Solanum lycopersicum L.)* is one of the most economically important vegetable crops worldwide. Fruit firmness is a target trait in tomato breeding because it assists transportation and storage. Small heat shock protein, also known as VISCOSITY1 (VIS1) gene, has been implicated in increasing the viscosity of fruit juice, early ripening and softening which emphasizes the importance of this gene in premature ripening. The aim of this study was, therefore, to develop a genome-edited tomato line with enhanced shelf life through CRISPR/Cas9 mediated knockdown of the VISCOSITY1 (VIS1) gene. The sgRNA for VIS1 gene was designed and, through Golden Gate ligation, constructed the CRISPR/Cas9 expression cassette carrying sgRNAs and the Cas9 gene driven by 35S promoter. The CRISPR/Cas9 expression Cassette was introduced into the tomato system through agrobacterium-mediated transformation, leading to precise mutation at target loci. For screening and confirmation of CRISPR edits, the integration of T-DNA through PCR by using Cas9 primers. T7 Endonuclease assay and sequencing further validated the mutation of the targeted gene. Functional characterization of edited tomato lines revealed delayed fruit ripening, reduced softening, and extended shelf life, representing the potential of **VIS1** knockdown in improving post-harvest traits without compromising fruit quality.

Keywords: *Tomato, CRISPR/Cas9, VISCOSITY1 gene, shelf-life.*

OP-04

Exploring Correlations of Key Nutritional Factors in Different Pakistani Wheat Varieties

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ABSTRACT

In Pakistan, evaluating wheat's nutritional quality is essential for addressing dietary gaps and deficiencies. Twelve Pakistani wheat varieties were examined to explore the correlations among key nutritional factors, including protein, amino acids, moisture, ash, starch, and fiber content. The correlation study identified six varieties with higher protein concentrations that generally had lower starch levels. In contrast, seven varieties exhibited an inverse relationship between protein concentration and fiber. Although the fiber content varied independently, a positive correlation between fiber and starch concentrations was observed in the two varieties. Variations in ash content did not clearly align with changes in other biochemical traits. Additionally, amino acid analysis revealed the highest concentrations in Narc-11 and TD-01, with lysine absent in all varieties, indicating a critical gap in the essential amino acids. SDS-PAGE protein profiling showed significant differences in glutenin and gliadin fractions, with Nawab and TD-01 exhibiting notable polymorphism and greater

gluten protein diversity. The correlation analysis concluded that wheat cultivars with richer amino acid profiles, such as TD-01 and Narc-11, had higher protein content combined with more diverse gluten proteins and moisture content. This analysis identified the potential of five wheat varieties (Narc-11, TD-01, Borlaug-16, Ujala-16, and FSD) as promising wheat varieties with better nutritional profiles.

Key Words: Wheat (Triticum Aestivum L.); Biochemical Traits; Amino Acids; Protein Content; SDS-PAGE; Nutritional Profiles; Correlation.

OP-05

Harnessing CRISPR-Cas9 to Remove β-Lactoglobulin: A Path to Allergen-Free Dairy

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ABSTRACT

Milk is an excellent source of nutrition, but certain proteins, such as β-lactoglobulin (BLG), α-lactalbumin, and casein, can trigger allergies in some individuals, particularly children. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system has emerged as a powerful tool for precise genome editing, including the modification of milk allergen genes. Using CRISPR/Cas9, researchers have successfully edited BLG genes in various dairy animals, such as cows, sheep, goats, and buffaloes. In bovine mammary epithelial cells (bMECs), the BLG knockout (BLG-KO) system was achieved using three single guide RNAs (sgRNAs) and a Casexpressing system delivered via electroporation. Western Blot analysis confirmed a significant reduction in BLG protein expression. In buffaloes, CRISPR facilitated bi-allelic editing (-/-) of the BLG gene, and somatic cell nuclear transfer (SCNT) produced BLG-edited embryos at the blastocyst stage. Similarly, in goats, one-cell stage embryos were co-injected with Cas9 mRNA and sgRNA to generate BLG-KO fibroblasts. These advancements demonstrate the successful application of CRISPR/Cas9 technology in producing β-lactoglobulin-free milk. Compared to traditional methods such as enzymatic hydrolysis (which is costly and may result in undesired epitopes) or Zinc Finger Nucleases (ZFNs) and TALEN-mediated editing (which are prone to offtarget effects), CRISPR/Cas9 offers a more efficient, precise, and cost-effective approach to eliminating milk allergens. This breakthrough holds significant promise for providing safer milk options for individuals with milk allergies.

Keywords: *CRISPR, BLG-KO, Allergen-Free Milk*

OP-06

Expression Analysis of ABCA1 in Type 2 Diabetic Pakistani Patients with and without Dyslipidemia and Correlation with Glycemic Index and Lipid Profile

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ABSTRACT

Diabetes Mellitus type II, earlier considered as an endocrinological disorder, is now regarded as an inflammatory disorder along with lipid aberrations. It demands regular monitoring, healthy dietary habits and lifestyle modification. This study focused on gene expression of ATP binding cassette protein 1 (ABCA1) in diabetic dyslipidemia patients in comparison with control groups of only diabetics and healthy individuals. Blood samples and data were collected from 390 recruited patients who were further divided into three groups (130 each). Glycemic index and lipid profile were assessed. Delta Delta Ct method was used that revealed downregulation of the studied gene more in diabetic dyslipidemia patients as compared to only diabetics and healthy controls. The Ct values of ABCA1 were associated with glycemic index and lipid profile using Pearson's correlation. A negative correlation with fasting blood sugar and a positive correlation with HbA1cwas observed in only diabetics group. While in diabetic dyslipidemia and normal healthy controls, a negative correlation was found with both. As far as the lipid profile is concerned a positive correlation was observed among only diabetics with whole lipid profile. In diabetics with dyslipidemia, a negative correlation with all parameters except the TAGs was observed. A positive correlation with all except HDL was observed in healthy controls. The Ct values and fold change were compared among diseased and healthy individuals by applying independent t-tests. The cycle threshold in only diabetics was $p = 0.000018$ and in diabetic dyslipdemia individuals was $p = 0.00251$, while fold change in only diabetics ($p = 0.000230$) and diabetics with dyslipidemia ($p = 0.001137$) was observed to be statistically significant.

Keywords: *ATP binding cassette protein 1 (ABCA1), Diabetic dyslipidemia, Expression, Delta delta CT method, Glycemic index, Lipid profile.*

OP-07

Mitigation of NSAID-Induced Enteropathy Through Dietary Psyllium Husk Supplementation in a Mouse Model

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed medications for pain management; however, prolonged use is associated with severe gastrointestinal complications, such as intestinal ulcers. This study evaluated the protective potential of psyllium husk, a well-characterized soluble dietary fiber, against NSAID-induced intestinal injury in a murine model. Enteropathy was

induced in mice via indomethacin (an NSAID), resulting in macroscopic intestinal edema, shortened intestinal length, and histopathological damage, including disrupted villi architecture. Molecular profiling using real-time PCR demonstrated (1) heightened inflammation, as evidenced by elevated TNF-α (a pro-inflammatory cytokine) and CXCL1 (a chemokine driving neutrophil recruitment); (2) oxidative stress, marked by upregulated glutathione peroxidase (GPX, an antioxidant enzyme) and inducible nitric oxide synthase (iNOS, a reactive nitrogen species generator); and (3) compromised mucosal barrier integrity, indicated by diminished expression of Mucin 2 (a critical mucus layer component) and Claudin (a key tight junction protein). These findings collectively underscore NSAID-driven pathological mechanisms involving inflammation, oxidative damage, mucus depletion, and epithelial barrier dysfunction.

Pretreatment with psyllium husk prior to NSAID exposure markedly attenuated these adverse effects. Gross and histological evaluations revealed preserved intestinal length, reduced edema, and restored villi morphology. Molecular analyses confirmed a significant reduction in TNF-α, CXCL1, GPX, and iNOS levels, alongside restored Mucin 2 and Claudin expression, reflecting diminished inflammation, oxidative stress mitigation, mucus regeneration, and enhanced epithelial integrity. These results highlight psyllium husk's multifaceted therapeutic potential in alleviating NSAID-induced enteropathy, positioning it as a clinically relevant, cost-effective dietary adjunct to enhance gastrointestinal safety in patients requiring long-term NSAID therapy.

Keywords: *NSAID-induced enteropathy, Dietary fibers, Psyllium husk.*

OP-08

Identification of Novel Genetic Variants Underlying Intellectual Disability in Pakistani Families

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ABSTRACT

Intellectual disability (ID) is a complex neurodevelopmental disorder affecting up to 2% of the global population, with significant social and economic implications. Despite its prevalence, the genetic basis of ID remains poorly understood, particularly in the local populations. We aimed to identify the genetic variants underlying ID in 15 recessive patterns of Pakistani families' pedigree. Using exome sequencing, we identified 15 variants in 13 genes, including five previously known (AGA, ASCC3, B4GALNT1, EIF3F, and GPT2) and seven novel Variants (CLDN11, TANC2, WRAP53, LAMB1, CC2D1A, VPS13B, and GPT2). Notably, two novel candidate genes, BFAR and CETN2, were discovered, highlighting the importance of exploring new genetic targets in ID. Variants were classified

according to the American College of Medical Genetics (ACMG) guidelines and predicted to have deleterious effects on protein function. Our study substantially expands the genetic landscape of intellectual disability (ID), underscoring the importance of genetic testing for early diagnosis, counseling, and therapeutic research. The discovery of novel genetic variants and candidate genes provides valuable insights into ID's molecular mechanisms, revealing potential therapeutic targets. These findings have significant implications for ID prevention, treatment, and support, highlighting the benefits of genetic testing in facilitating early diagnosis and tailored interventions.

Keywords: *Intellectual disability, Neurodevelopmental disorder, Exome sequencing, Pakistani families.*

OP-09

Mutation in Superoxide Dismutase Gene (SOD_2) and its Relation with Oxidative Stress in Hypertension

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ABSTRACT

Hypertension is a chronic condition characterized by the persistent elevation of systemic arterial pressure, significantly increasing the risk of cardiovascular disease, kidney failure, stroke, and mortality. The superoxide dismutase 2 (SOD_2) gene play a crucial role in oxidative stress regulation, a key factor in hypertension pathogenesis. This study investigates SOD_2 gene expression, mutations, and their impact on oxidative stress, while also assessing lifestyle, genetic predisposition, and metabolic risk factors in hypertensive individuals. Blood samples and demographic data were collected from hypertensive and normotensive individuals following American College of Cardiology (ACC)/American Heart Association (AHA) criteria. Antioxidant activity was measured through biochemical assays, and DNA extraction, PCR-RFLP, and sequencing were used to analyze allelic and genotypic frequencies, amino acid substitutions, haplotypes, and linkage disequilibrium.For gene expression analysis, RNA was extracted using Trizol reagent, followed by RT-PCR and cDNA synthesis. Forward and reverse primers were designed using the human SOD_2 nucleotide sequence from NCBI. Transfection studies were performed using an appropriate cell line to investigate SOD_2 mutations. Additionally, seven variant constructs with single amino acid substitutions in the SOD_2 signal sequence were generated to assess their effects on gene expression and reactive oxygen species (ROS) production. Oxidative stress levels were measured using quantitative and qualitative assays, and the most functionally significant variant was further analyzed via Molecular Mechanics/Dynamics

(MMD) simulation. This study enhances the understanding of SOD_2 gene mutations and oxidative stress mechanisms in hypertension, providing potential insights for biomarker development and early disease detection.

Keywords: *Antioxidant genes, SOD_2 gene, Mitochondrial targeting signal, rs4880, Antioxidant enzyme, Oxidative stress.*

OP-10

Novel Sequence Variants in the SLC9A7 and IQSEC2 Genes in a Family with X-Linked Intellectual Disability

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ABSTRACT

Intellectual disability (ID), characterized by delayed development of cognitive, language, motor, and social skills, is a complex condition influenced by both genetic and environmental factors. In this study, we investigated a family from Peshawar, Khyber Pakhtunkhwa, Pakistan, using whole exome sequencing (WES) to identify the molecular causes of ID. We discovered two novel hemizygous missense variants in the X-chromosome: c.2680G>A (p.Asp894Asn) in the IQSEC2 gene and c.1207T>A (p.S403T) in the SLC9A7 gene. Segregation analysis confirmed the X-linked inheritance pattern of these variants, with affected individuals (II-3, II-4, II-5) exhibiting hemizygosity. Further structural analysis of the IQSEC2 and SLC9A7 mutation using 3D modeling revealed potential changes in the protein structures due to the missense variants. These findings contribute to a better understanding of the genetic underpinnings of intellectual disability and emphasize the importance of genetic screening for early diagnosis and personalized treatment, particularly in consanguineous populations.

Keywords*: Neurogenetics, Rare diseases, Pakistan, Cousin marriages, Novel.*

OP-11

The Role of Enzymes in Biochemical Catalysis Advances and its Applications

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ABSTRACT

Enzymes are pivotal in biochemical catalysis, driving reactions essential for life and numerous industrial processes. This review explores the fundamental principles of enzyme catalysis, including their structure, mechanisms of action, and factors influencing activity. Advances in enzyme engineering, such as site-directed mutagenesis, directed evolution, and computational approaches,

have revolutionized the field, enabling the development of enzymes with enhanced efficiency, specificity, and stability. High-throughput screening and metagenomics have further facilitated the discovery of novel enzymes, while advanced techniques like X-ray crystallography and cryo-EM provide deeper mechanistic insights. The applications of enzyme catalysis are vast, spanning industries such as pharmaceuticals, food processing, biofuels, and environmental sustainability. Enzymes are employed in drug synthesis, chiral compound production, waste management, and green chemistry initiatives, showcasing their versatility and ecological benefits. Despite these advancements, challenges such as stability under extreme conditions, substrate specificity, and production costs remain significant barriers. Looking forward, emerging technologies like synthetic biology, nanotechnology, and artificial intelligence offer promising solutions to overcome these limitations and expand the potential of enzyme applications. This review highlights the transformative impact of enzymes in science and industry, emphasizing their continued importance in advancing sustainable and innovative solutions.

Keywords: *Enzymes, Biochemical, Catalysis, Advances, Applications*

OP-12

Unlocking the Potential of *Thermogutta terrifontis*: Tt_End5A, a Multifunctional Enzyme for the Bioethanol Industry

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ABSTRACT

The enzymatic saccharification of plant biomass polymers, such as β-glucans, celluloses, and xylans, is crucial to produce biofuels and valuable products. To this end, we characterized a novel, processive, and multifunctional endo-1,3-1,4-β-D-glucanase (Tt_End5A) from the hyperthermophilic bacterium Thermogutta terrifontis. Tt_End5A exhibited a broad substrate specificity, efficiently degrading various β-polysaccharides, including barley glucan, lichenan, and xylan, at optimal temperatures (70-80°C) and pH 7. Notably, the enzyme demonstrated exceptional thermal stability, retaining over 90%

activity at 80°C, and high processivity on regenerated amorphous cellulose (RAC). The 1.20 Å crystal structure of the Tt_End5A catalytic domain revealed a unique glycoside hydrolase family 5 (GH5) fold, featuring a catalytic TIM-$(\beta/\alpha)_8$-barrel supplemented with additional β-strands, elongated α-helices, and a rare cis-non-Pro (His481-cis-Ala482) peptide. This distinctive architecture is likely linked to the enzyme's multifunctionality and processivity. A large central cleft was observed in the 3D structure, which may facilitate substrate binding and catalysis. A novel N-terminal multivalent carbohydrate-binding module (CBM) was identified, enhancing the enzymatic degradation of soluble and insoluble polysaccharides. Mutagenesis and ligand interaction studies elucidated the catalytic mechanism, implicating residues E329 and E448 as the proton donor and nucleophile, respectively. The multifunctional and processive nature of Tt_End5A makes it an attractive candidate for reducing the enzymatic complexity of biomass saccharification, with potential applications in the bioethanol, pharmaceutical, feed, and food industries.

Keywords: *Structural biology, Multifunctionality, Processivity, Biomass polysaccharides, Biocatalysis, Endoglucanase.*

OP-13

Industrial Potential of Recombinant Manganese-Catalases from Geobacillus thermopakistaniensis for Sustainable Textile Bleach Clean-Up

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ABSTRACT

Catalases play a crucial role in the textile industry by efficiently removing hydrogen peroxide residues from bleached cotton fabrics. However, commercially available catalases, primarily heme-catalases and catalase-peroxidases, are thermolabile, limiting their industrial efficiency. This study explores the potential of three recombinant manganese-catalases (Cat_{Gt}, Cat-II_{Gt}, Cat_{Gt}-ΔC) from Geobacillus thermopakistaniensis as thermostable alternatives for bleach clean-up. Among them, Cat-II_{Gt} exhibited the highest activity, remarkable thermostability, and alkaline tolerance, making it particularly suitable for direct application in bleach baths. Its integration into textile processing reduces water consumption while maintaining process efficiency. To the best of our knowledge, this is the first study highlighting the application of manganese-catalases—an underexplored enzyme class—in the textile industry, offering a sustainable and eco-friendly enzymatic solution.

Keywords: *Manganese-Catalases; Textile Industry; Thermolabile Enzyme; Cat_{Gt}; Geobacillus thermopakistaniensis.*

OP-14

Bioconversion of Starchy Food Waste into Maltose and Glucose Syrups Using *Thermococcus kodakarensis* Pullulan Hydrolase

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ABSTRACT

Food waste poses a significant global challenge, with approximately 1.3 billion tons discarded annually, accounting for one-third of total food production, according to the Food and Agriculture Organization (FAO) of the United Nations. Beyond contributing to environmental pollution, food waste also facilitates extensive fungal growth, threatening public health. In this study, we utilized various starchy food wastes, including undersized potatoes, chapati, bread powder, broken rice, rice powder, and corn powder, as substrates to produce maltose and glucose syrups. This was achieved using pullulan hydrolase from Thermococcus kodakarensis (TK-PUL). The novelty of our approach lies in three key aspects: (i) the enzymatic hydrolysis was conducted without the use of liquefying amylase, (ii) starch pre-gelatinization was not required, and (iii) undisrupted E. coli cells expressing the TK-PUL gene served as the enzyme source, leveraging the extreme thermostability of TK-PUL. Since glucose and maltose are key precursors for a variety of biotechnological applications, our study highlights a cost-effective and sustainable strategy for converting starchy food waste into valuable biochemical products. This approach not only extracts useful compounds from discarded food but also contributes to environmental sustainability by reducing food waste-related pollution.

OP-15

Pcal_0976, a Pullulanase Homologue from *Pyrobaculum calidifontis,* Shows Unique Intragenomic Evolution and Substrate Specificity

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ABSTRACT

The present study aimed at evolutionary analysis and recombinant production of a novel protein, Pcal_0976, annotated as pullulanase in the genome sequence of Hyperthermophilic archaeon *Pyrobaculum calidifontis*. The motif search showed two glucodextran_C-like domains and a domain of unknown function (DUF4134) in Pcal_0976. Detailed evolutionary analysis revealed that the glucodextran_C-like domains in Pcal_0976 have evolved from a single glucodextran_C domain present in another pullulanase (Pcal_1616) in the same organism. The recombinant production of Pcal_0976 in Escherichia coli resulted in an insoluble and inactive protein, which was solubilized

using guanidine hydrochloride and refolded in an active form in the presence of arginine. Refolded Pcal_0976 displayed hydrolysis of glycogen, dextran, dextrin, and starch. No hydrolytic activity was detected against pullulan. These results indicate that Pcal_0976 may not be a pullulanase but a novel glycoside hydrolase that emerged during the evolution of *P. calidifontis*.

Keywords: *protein evolution, glycoside hydrolase, glucodextran C domain.*

OP-16

From Waste to Value: Valorization of Food Wastes Through Enzymatic Processing

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ABSTRACT

Food and Agriculture Organization (FAO) of the United Nations estimated that about one third of the total food produced for human consumption is wasted every year. To feed around 8 billion globe population, the only production of more and more food crops will not be sufficient. Besides conservation of food resources, we need to devise strategies for innovative reprocessing of food wastes. We have characterized a novel thermo-acidophilic pullulanase from Thermococcus kodakarensis (TK-PUL) which can convert wasted foods like undersized tubers, damaged grains and left over chapatti into sweetener syrups. The beauty of this enzyme is its versatile nature. It can perform efficiently under extreme conditions and hydrolyzes purified, raw as well as crude starches. Besides its purified form TK-PUL may also be used in the form of crude preparation or as partially purified enzyme. Wasted foods (undersized potatoes, damaged grains and chapatti) were valorized by TK-PUL into maltose rich syrups. Conversion of higher saccharides into glucose was achieved with the help of amyloglucosidase (glucoamylase from Aspergillus niger). Glucose and maltose syrups are widely employed in a range of food industries like confectionery, baking, beverages etc. These syrups are mixtures of fermentable sugars and can serve as precursors for a variety of biotechnological products like organic acids, ethanol, amino acids etc. We have also devised cost effective strategies for local production of TK-PUL. Comparative studies using commercially available industrial enzymes were also carried out. We have found that TK-PUL works as good as its commercial counterparts. Cheaper production of local enzyme (TK-PUL) for application in valorization of food wastes will indirectly help in economical control environmental pollution.

Keywords: *Pullulanase, valorization, glucose syrup, maltose syrup, Thermococcus kodakarensis.*

OP-17

A track for the district Sialkot, Pakistan, to Achieve the World Health Organization's HCV Elimination Plan of 2030

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ABSTRACT

Liver cirrhosis is caused by the blood-borne infection Hepatitis C Virus (HCV), which damages hepatic (liver) cells. Sixty-seven subtypes and twenty provisional subtypes comprise the seven genotypes (GT) of the hepatitis C virus. Approximately 10 million people in Pakistan are afflicted with HCV, making it the second most contaminated country after Egypt. One in twenty persons in Pakistan is infected with the Hepatitis C virus. Our objective is to find out either District Sialkot, Punjab Pakistan is on track of World Health Organization 2030 HCV elimination plan or not. This study was conducted from 1st April 2016 to 31st August 2024 in this HCV RT-PCR (Both Qualitative and Quantitative) data was collected from Dr. Abdul Sattar Lab® after written permission from its R&D department. In our research work total sample size n-22,015 (Margin error 1%, Confidence level (C.L) 99%, Z score 2.98 at 99% C.L) was taken. Seasonal Auto-Regressive Integrated Moving Average (SARMA) model (SARIMA (0, 1, 1), (0, 1, 1)6) was used to check District Sialkot HCV elimination track by using Jupyter Notebook of Anaconda Navigator 1.9.12. This study concluded that in District Sialkot, HCV infected male percentage is high with a higher viral load than females. Seasonal Auto-Regressive Integrated Moving Average (SARIMA) shows effective and optimized modelling and determined that District Sialkot is not on track to achieve the WHO HCV elimination plan and before 2030. In District Sialkot Government and NGOs should revise their policies and speed up their work plan to elimination HCV from this region.

Keywords: *HCV, SARIMA model, WHO HCV elimination plan*

OP-18

Nitrogen-Dependent Diversity Dynamics of Cultivable Methanol-Utilizing Bacterial Community in Rice Paddy Bulk and Rhizospheric Soils

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ABSTRACT

Methanol, the second most abundant volatile organic compound released by plants, plays a crucial role in atmospheric chemistry. Environmental methanol emissions result from both biochemical and anthropogenic activities, including industrial processes, biomass combustion, and agricultural

practices. Methanol-utilizing bacteria (methylotrophs) mitigate these emissions by degrading methanol, contributing to the carbon cycle and promoting plant growth. However, their diversity remains underexplored in Pakistani soils due to cultivation challenges. Therefore, the current study designed to investigate the diversity of cultivable methanol-utilizing methylotrophs in rice paddy fields, hypothesizing that distinct communities are enriched in soil and rhizosphere environments under varying conditions. Bacteria were isolated using different nitrogen sources and temperatures, and whole-genome sequencing was performed to assess their plant growth-promoting potential. The findings revealed that Methylobacterium, Ancylobacter, Achromobacter, Xanthobacter, Moraxella and Klebsiella were more prevalent in nitrate-based media at 30°C, whereas ammonium-based media primarily supported Hyphomicrobium and Methylobacterium. At 45°C, Hyphomicrobium was enriched in nitrate-based media, while ammonium-based media favored Brevibacillus. Further characterization based on growth patterns and carbon-substrate utilization highlighted their ecological role in environments affected by nutrient variability and temperature fluctuations. Whole-genome sequencing of Methylobacterium sp. TS1 and Hyphomicrobium sp. TRA1 revealed versatile methanol metabolism and plant growth-promoting traits, including phosphate solubilization, tryptophan-dependent biosynthesis, and the production of ACC deaminase, siderophores, and antimicrobial compounds. Conclusively, the study provides insights into methanol mitigation in terrestrial environments while emphasizing the potential of plant growth-promoting methylotrophs as sustainable alternatives to chemical fertilizers and pesticides.

Keywords: *Methanol mitigation, methylotrophs, rice paddy soils, plant-growth promoting methylotrophs.*

OP-19

Development of Microbial Organic Fertilizer using Pilot Scale Solid State Fermenter

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ABSTRACT

Microbial Organic Fertilizer was developed using different organic waste substrates which carry the microbial consortium within themselves. The important substrates used were wheat straw, starch waste from potato industry waste, litter leaves, and diluted molasses to fortify the growth. To make the process work faster, bacterial and fungal inoculum was also added to the Pilot Solid State Fermenter of 500 kg level at PCSIR, Lahore. It was an automated system in which low pressure air was introduced to make it semi aerated as the air was allowed to pass for couple of hours in the morning and evening. Under optimal conditions of moisture 30 %, the temperature was allowed to fluctuate as at the

beginning mainly mesophilic bacteria flourished, whereas, after 3-4 days the temperature rose steadily from room temperature which was around 30 ℃ to 45 ℃ and above. Similarly, the rotation of the reactor was carried out after every 24 hours for mixing of substrates and microbial community. Thermophilic fungi flourished under these conditions and the breakdown of organic material was faster which turned the organic load converted into 10 days from greenish brown to dark brown, black. The Organic fertilizer was analyzed for moisture, pH, carbon, nitrogen, phosphorous, etc. Evaluation of fertilizer was also carried out at a couple of places for efficacy.

OP-20

Bacterial Cell-Based Bioremediation of Industrial Wastewater: Isolation and Characterization

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ABSTRACT

Industrial waste effluents laden with heavy metals and other dying agents are a leading cause of environmental pollution. Chromium (Cr^{+6}), used in the tanning process, is the most common polluting agent released from the leather industry. Untreated tannery effluents are badly affecting nearby land and water bodies as well as human health, particularly in industrial areas (Lahore, Sialkot, Karachi etc.). In this study, Cr-resistant bacteria isolated from the cable industry Kot Lakhpat Lahore were identified on a molecular basis and assessed for their ability to degrade waste sludge. Amongst these, a Cr-resistant bacterium *Bacillus cerus* exhibited 35mM minimum inhibitory concentration in $K_2Cr_2O_7$ supplemented media with optimal growth at 37 °C and 7 pH. The bacterial strain was also capable of thriving in the presence of various other heavy metal salts ($ZnCl_2$, $CdSO_4$, $NiSO_4$ and $HgCl_2$), highlighting its survival in harsh environmental conditions and their reduction. The Cr^{+6} reduction capacity of the bacteria was 35.5% in culture supernatant and 8.2% in cell lysate, which showed a reduction in initial Cr concentration (2 mg/L) in 45 minutes, 37 ºC. The bacteria culture 10% (v/v) also showed good potential to decolorize azo dyes compounds in textile wastewater and this biosorption capacity after 24, 48, and 72 hours were 29%, 57%, and 81%, respectively. This treated textile wastewater was further examined for Triticum aestivim (wheat) seed's germination capacity as an indication of phytotoxic effect of textile mills effluents. With treated water, a 50% increase in seed radicle and plumule length was observed in comparison to untreated water. These findings emphasize its potential in environmental cleanup, particularly in biosorption of heavy metals and water decolorization. Resistant bacteria could be a potential candidate for waste-water detoxification as an inexpensive, environmentally friendly approach and conservation of our natural flora.

Keywords: *Industrial wastewater, microbial remediation, biosorption, phytoxicity.*

OP-21

Enhancing Solubility and Stability of *Pyrococcus abyssi* Amylase-Catalytic Domain through SUMO Fusion for Industrial Applications

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ABSTRACT

The solubility and stability of industrial enzymes are crucial factors that influence their efficacy in various biotechnological applications. This study investigates the effect of Small Ubiquitin-like Modifier (SUMO) fusion on the solubility, stability, and catalytic efficiency of the amylase-catalytic domain from *Pyrococcus abyssi*. The SUMO fusion strategy significantly enhanced enzyme solubility, leading to a 1.5-fold increase in enzymatic activity, particularly against glycogen and amylopectin. A comparative analysis of enzyme activity under different conditions revealed that metal ions and chemical reagents substantially modulate catalytic performance, underscoring the biochemical versatility of the SUMO-fused amylase. The resulting improvements in solubility and activity suggest that SUMO fusion mitigates common challenges associated with recombinant enzyme production, facilitating more efficient expression and functional stability. Given the enzyme's enhanced characteristics, it holds promising potential for industrial applications in food processing, textiles, pharmaceuticals, and animal feed. This study offers crucial insights into the molecular strategies for optimizing enzyme performance, thereby paving the way for the development of more robust and efficient biocatalysts. The findings highlight SUMO fusion as a viable approach for improving enzyme-based industrial processes, offering a sustainable and innovative solution for enzymatic applications in diverse sectors.

Keywords: *α-Amylase Catalytic domain, Pyrococcus abyssi, Small Ubiquitin Modifying Protein (SUMO).*

OP-22

Mosquitocidal Potential of a Novel Surface Layer Protein from *Geobacillus thermopakistaniensis MAS1*

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ABSTRACT

Mosquitoes act as primary vectors for transmitting arboviruses and protozoan parasites, underscoring the urgency to develop sustainable strategies for vector control. Bacterial larvicides offer a viable solution, combining cost-effectiveness, minimal environmental impact, and scalability. In this study, we evaluated the larvicidal potential of *Geobacillus thermopakistaniensis* MAS1 by isolating and characterizing its surface-layer (S-layer) protein. This protein is a structural component of cell envelopes and protects bacteria from harsh environmental conditions. A rapid lithium chloride (LiCl) extraction protocol was optimized to extract S-layer proteins efficiently, yielding 53 mg of protein per liter of bacterial culture. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showed about 100 kDa band which was subjected to bottom-up proteomics analysis. The peptide mass fingerprints obtained from proteomics correspond to a hypothetical protein annotated in the *G. thermopakistaniensis MAS1* genome having 22 % sequence coverage. Bioinformatics analyses confirmed its non-cytoplasmic localization alongside conserved domains characteristic of S-layer proteins in Gram-positive bacteria. Structural characterization revealed a composition of 10% α-helices and 55% β-sheets, consistent with the β-sheet-rich architecture typical of Slayer proteins. Three-dimensional modeling using I-TASSER, Phyre2, and AlphaFold2 further corroborates structural homology to known S-layer frameworks. Insecticidal assays demonstrate dose-dependent toxicity against Culex quinquefasciatus (LC50= 6.84 µg/mL) and Aedes aegypti (LC50= 20.86 µg/mL) larvae, indicating species-specific efficacy. In contrast, Anopheles stephensi exhibited no mortality even at 60 µg/mL, suggesting selective activity. These findings highlight the S-layer protein of *G. thermopakistaniensis MAS1* as a targeted, eco-friendly larvicide candidate against Aedes and Culex species, which are major vectors of dengue, Zika, and West Nile viruses. The optimized LiCl extraction method enhances scalability for potential field applications. Further studies are needed to unravel the mechanistic basis of its toxicity and evaluate resistance development in target species.

Keywords: *Aedes, bottom-up proteomics, Culex, Geobacillus thermopakistaniensis MAS1, Larvicidal activity, MALDI-TOF, S-layer protein*

OP-23

Genetic Engineering and Characterization of Human Serum Amyloid A (SAA)

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ABSTRACT

Genetic engineering and characterization of serum amyloid A (SAA), involved in neurodegenerative diseases, is important to explore the pathophysiological mechanism of fibrillation. The present study

deals with the engineering and characterization of human SAA. Mature human SAA gene (318 bp) was sub-cloned in expression vectors pET-which was analyzed by double digestion using NdeI/HindIII, and confirmed by Sanger sequencing, followed by the expression in Escherichia coli Rosetta (DE3) pLysS. This construct gave significant expression at 30 °C with 8 hours post-induction using 0.1mM IPG, yielding ~ 50 OD_{280} soluble protein from 1L of culture. The molecular weight of SAA was 11.8 kDa. Since SAA recombinant protein was in soluble form, it was precipitated down by 1M ammonium sulphate. 150 OD_{280} of soluble dialysed SAA protein solution was purified by urea-sepharose column, eluting the major fractions of SAA in 0.2 – 0.6M NaCl gradients, giving 82% yield of purified SAA as shown by 15% Tricine-PAGE. SAA was also purified by Q-sepharose column which gave relatively low yield of about 50%. This feature is attributed to the hexameric oligomer nature of SAA and that is why under denaturing conditions in urea-sepharose column, yield was higher as compared to Q-sepahrose column. Purified SAA was characterized by circular dichroism spectroscopy showing 86% helical and 2% beta regions. Under optimised in vitro physiological conditions, recombinant human SAA fibrils showed significant binding with serum amyloid P-component (SAP) in the presence of 2mM calcium ions. The present study highlights that the fibrils formed from recombinant SAA exhibited similar features as that of native fibril-SAP complex in neurodegenerative and amyloidogenic diseases. Future work includes inducing fibrillation in animals and mammalian cell-lines, investigating the genes involved in up- and down-regulation of fibrillation phenomenon.

Keywords: *Serum amyloid A, Fibrils, Purification, Amyloidosis, Neurodegenerative diseases.*

OP-24

Probing The Functional Significance of T263 Residue In The Regulatory Site of Recombinant L-Asparaginase: AfASNase

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ABSTRACT

L-Asparaginase (EC 3.5.1.1) role as an anticancer drug and acrylamide mitigator in the food industry is noteworthy. It catalyzes the deamination of L-Asparagine into L-Aspartic acid and ammonia. Bioinformatic studies indicated that the regulatory site of the recombinant L-Asparaginase from Anoxybacillus Sp. was formed by T262, T263, C265, G269 and Thr294 residues. To investigate the role of T263 amino acid in the enzyme function, site-directed mutagenesis was performed, and the resulting enzyme variant was analyzed for the changes in its biochemical characteristics. By using Phusion polymerase and PCR technology the replacement of L-Threonine with L-Alanine at 263rd position was achieved in the native enzyme. The T263A-AfASNase mutated plasmid was transformed

into E. coli BL21 cells. The enzyme variant gave soluble expressions and after partial purification it presented ~38kDa size on SDS-PAGE. Biochemical analysis demonstrated that the mutated AfASNase variant exhibited a 20% reduction in enzymatic activity compared to its native counterpart. The mutant enzyme optimally worked at 60ºC and pH 7. However, there was a significant reduction in thermostability at 60°C, with the variant maintaining activity for 1.5 hours, whereas the native enzyme remained active for 3.5 hours. The enzyme variant also displayed activity with D-Asparagine, L-Glutamine, and D-Glutamine, in addition to L-Asparagine, that was the sole substrate for AfASNase. The computational analysis revealed that the substitution of a hydrophilic L-Threonine with a hydrophobic L-Alanine enhanced the structural stability of the variant enzyme, as evidenced by the reduced root mean square deviation (RMSD) values. Therefore, the findings from this research study provided novel insights into the functional role of T263 residue in the regulatory site of AfASNase and elucidated the impact of its substitution on the biochemical features and enzyme activity. These findings provide valuable insights into the structure-function relationships of AfASNase and highlight the potential of protein engineering in designing enzymes with tailored properties.

Keywords: *Regulatory site, site-directed mutagenesis, computational analysis, phusion polymerase and PCR technology.*

OP-25

Insights into The Differential Proteome Landscape of A Newly Isolated Paramecium Species in Response to Metal Stress

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ABSTRACT

Microbial-mediated bioremediation has a substantial potential to successfully restore the polluted environment. However, limited understanding of the underlying mechanisms hampers the implication of microbial-mediated bioremediation. The emergence of transcriptomics, proteomics, and metabolomics (referred to as OMICS) at the whole genome level represents a promising toolkit to address these questions. Here, a mass spectrometry-based quantitative proteome profiling approach was conducted to explore the differential protein levels in cadmium-treated *Paramecium multimicronucleatum*. The Proteome Discoverer software was used to identify and quantify differentially abundant proteins. The proteome profiling generated 7,416 peptide spectral matches, yielding 2824 total peptides, corresponding to 989 proteins. The analysis revealed that 29 proteins

exhibited significant ($p \leq 0.05$) differential levels, including a higher abundance of 6 proteins and reduced levels of 23 proteins in Cd2+ treated samples. These differentially abundant proteins were associated with stress response, energy metabolism, protein degradation, cell growth, and hormone processing. Briefly, a comprehensive proteome profile in response to cadmium stress of a newly isolated Paramecium has been established that will be useful in current and future studies to identify critical components involved in the bioremediation and detoxification of metal ions in the environment.

Keywords: *Bioremediation; Cadmium; Metabolism; Paramecium; Proteins.*

OP-26

Molecular Construction and Engineering of a *Pyrococcus abyssi* Sourced Lysophospholipase for Enhanced Stability and Activity

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ABSTRACT

Lysophospholipases (LPLs) belong to α/β hydrolase family. Conserved GXSXG motif and a catalytic triad (Ser88, Asp204 and His234) are the distinguishing features of LPLs. LPLs (EC 3.1.1.5) find their tremendous applications in food industry (degumming of edible oils and processing of starch hydrolysates). The present research work aims at engineering of an LPL (Pa-LPL) from *Pyrococcus abyssi* to improve its activity and stability. Purified recombinant metal ion-independent Pa-LPL (~31 kDa) displayed its peak activity at 65 °C and pH 6.5 with half-life ($t_{1/2}$) of 1 h at 95 °C. To improve thermostability of Pa-LPL without compromising its activity, two protein-engineering approaches were employed: rigidifying flexible sites (RFS) through truncation of flexible loop (50-61) and SUMO (small ubiquitin-related modifier) fusion technology. A truncated version of Pa-LPL (t-LPLΔ12) was developed through overlap extension (OE) PCR. Heterologous production of t-LPLΔ12 (~31 kDa) was achieved in E. coli. Purified truncated enzyme worked optimally at 65 °C and pH 6.5. In comparison to Pa-LPL, 1.9-fold enhancement in $t_{1/2}$ of t-LPLΔ12 was observed at 95 °C. However, 1.1-fold decrease in V_{max} of t-LPLΔ12 was noted towards hydrolysis of p-nitrophenyl butyrate relative to wild type Pa-LPL. Thereafter, SUMO fusion technology was used for improving the stability of Pa-LPL without undermining its catalytic activity. To produce SUMO-fused Pa-LPL (6H-S-PaLPL), Pa-LPL was cloned and expressed in pET28a-SUMO vector. Purified recombinant 6H-S-PaLPL (~41 kDa) exhibited its optimum activity at 35 °C and pH 6.5 with 2.4-fold increase in V_{max} of 6H-S-PaLPL towards p-nitrophenyl butyrate hydrolysis. Moreover, $t_{1/2}$ values for Pa-LPL and 6H-S-PaLPL were calculated to be 1 and 12 h, respectively at 95 °C. To conclude, SUMO fusion technology was found

to be a promising strategy to improve stability of Pa-LPL without compromising its activity for enhancing its practical utility.

Keywords: *Pyrococcus abyssi, lysophospholipase, truncation of flexible loop, SUMO fusion, improved properties.*

OP-27

Recombinant Production of a Thermostable Pectinase: Challenges and Strategies

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ABSTRACT

Pectinases are enzymes involved in the degradation and modification of pectin. Pectinases can be obtained from plants, fungi, bacteria, and yeast. Pectinases have applications in many industrial processes, such as fruit juice clarification and processing, biomass degradation, fiber, and the paper industry. These processes require thermostable pectinase that can sustain high temperatures and acidic environments. This study aims to produce a thermostable form of pectinase through in-silico design and recombinant DNA technology. The genome of thermophiles (Thermotoga species) was screened for pectinase genes. Structural insights were analyzed using molecular modeling and ligand docking tools to understand key functional determinants, and protein engineering approaches were applied to design variants with enhanced enzyme properties for improved industrial suitability. In this study, the wild-type and truncated forms of pectate lyase were cloned and expressed in Escherichia coli to achieve high-yield recombinant production. Expression conditions were optimized for maximal protein solubility and stability. The recombinant enzymes were successfully purified, and their characterization is currently in progress. This includes evaluating enzymatic activity, thermostability, acid resistance, and hydrophobicity. This optimized enzyme will be further investigated for its potential applications in food processing, juice clarification, and lignocellulose hydrolysis, particularly in combination with other hydrolytic enzymes to enhance biomass degradation efficiency. Further, more variants will be designed and produced, and the most promising variant will be selected based on acid stability and thermostability compared to the wild-type enzyme. The study aims to contribute to the development of robust biocatalysts for industrial processes requiring efficient pectin degradation under extreme conditions.

Keywords: *Thermostable pectinase, Recombinant production, Food processing.*

OP-28

Structural Insights into Ral GTPase Membrane Dynamics and their Role in Colorectal Cancer Progression

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ABSTRACT

RalA and RalB, small G-proteins within the Ras superfamily, share ~85% sequence identity but play distinct roles in normal cellular function and tumorigenesis. This study investigates the structural and functional differences between RalA and RalB, focusing on RalB's unique insertion at position 116 and its impact on effector binding. Additionally, we explore the interaction between RalA's C-terminus and its G-domain, as well as its binding to calmodulin, which may regulate membrane association. The role of RalA and RalB in colorectal cancer (CRC) was also examined, given their potential involvement in KRAS-driven tumorigenesis. NMR spectroscopy was used to analyze ^{15}N relaxation data from T_1, T_2, and NOE experiments to assess the dynamic properties of the G domains. The structural analysis of calmodulin bound to the lipidated C-terminal tail of RalA revealed a mechanism for its regulation of membrane dynamics. Additionally, immunohistochemical analysis of 123 CRC tissue samples assessed RalA and RalB expression levels and their correlation with clinicopathological features. This study presents the first solution structure of RalA and highlights key differences between RalA and RalB. Structural analysis showed that RalB's insertion at position 116 affects switch regions, influencing effector binding and tumorigenic potential. ^{15}N HSQC spectra of RalA revealed exchange broadening at the C-terminus, suggesting intramolecular interactions, confirmed by salt titration experiments. CRC samples (mean age: 47.66 years, male predominant) were mostly diagnosed at advanced stages (III/IV). Immunohistochemistry showed higher RalA (IRS 6) and RalB (IRS 4) expression in tumors, with RalB showing greater variability. High RalA/RalB expression correlated with poor survival. Weak associations were observed between RalA/RalB expression and clinical features, with RalA positively linked to age and RalB negatively correlated with stage and grade. These findings provide insights into Ral GTPase regulation and highlight their prognostic potential in CRC.

Keywords: *RalA, RalB, KRAS, Colorectal Cancer, Membrane Dynamics, NMR Spectroscopy.*

OP-29

Advancing Theranostic Nanomedicines for Targeted Therapy in Gastrointestinal Cancer

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ABSTRACT

Gastrointestinal (GI) tract cancer remains one of the leading causes of mortality globally, necessitating advanced therapeutic strategies. Theranostic nanomedicines, which combine therapeutic and diagnostic functions, offer a promising solution for targeted drug delivery and real-time imaging in cancer treatment. The design and application of various nanovehicles—specifically quantum dots (Zn-based), gold nanoparticles (GNPs), and carbon-based nanomaterials—for the effective targeting and treatment of GI tract cancer is a very important step in designing theranostic nanomedicines. The nanomedicines utilize pH-sensitive bonds, optimizing drug release within the acidic tumour microenvironment of the GI tract. Zinc-based quantum dots, gold nanoparticles, and carbon nanodots were synthesized, functionalized, and conjugated with chemotherapeutic agents, such as doxorubicin, for controlled drug release. Each nanovehicle was evaluated for its biocompatibility, targeting efficiency, and imaging capabilities. The pH-dependent release profiles were tested in vitro, and cytotoxicity assays demonstrated prolonged drug activity while minimizing off-target effects. In vivo studies confirmed the successful targeting of cancer cells and the enhanced therapeutic efficacy of the nanomedicines. Our results showed that zinc, gold and carbon-based nanomedicines provided superior fluorescence imaging. At the same time, quantum dot formulations excelled in diagnostic and therapeutic roles due to their unique optical properties. These findings highlight the potential of pH-responsive nanomedicine designs for improving the treatment of GI tract cancers while offering real-time monitoring capabilities. This research paves the way for future clinical applications of theranostic nanomedicines in oncology, particularly for patients with GI tract malignancies, by enabling precise treatment and minimizing systemic side effects.

Keywords: *Theranostic Nanomedicines, Targeted therapy, nano-vehicle, gastric cancer, colon cancer*

OP-30

Tracking Molecular Response of Tyrosine Kinase Inhibitors Therapy in Chronic Myeloid Leukemia Patients Using RT-PCR Profiling of BCR-ABL Transcripts

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ABSTRACT

Chronic myeloid leukemia (CML) is a hematological malignancy caused by the reciprocal translocation between chromosomes 9 and 22, forming the Philadelphia (Ph) chromosome. This results in the bcr-abl oncogene, which encodes a chimeric protein that activates signalling pathways like PI3K, MAPK, and JAK/STAT, promoting leukemogenesis and reactive oxygen species (ROS) production. CML progresses through three phases: chronic, accelerated, and blast. Tyrosine kinase inhibitors

(TKIs), including first-generation imatinib and second-generation dasatinib and nilotinib, are the primary treatment options. However, resistance to TKIs remains a significant challenge, potentially leading to disease progression. In cases of TKI intolerance or resistance, bosutinib and ponatinib are prescribed. The bcr-abl oncogene can be detected through techniques such as PCR and FISH. Imatinib has shown remarkable efficacy, but its discontinuation often leads to disease relapse. Ongoing research aims to improve therapeutic strategies and address resistance mechanisms. The experimental work was conducted in the Advanced Biochemistry Lab, Department of Basic and Applied Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore, following ethical approval. Diagnosed CML patients of both genders aged above 18 years were included, while patients with myeloma, AML, pregnancy, or other chronic medical conditions were excluded. This study analyzed specific BCR-ABL transcript variants in 30 Pakistani CML patients to detect common fusion protein isoforms. RNA extraction, cDNA preparation, primer design and real-time PCR were utilized to profile three transcript variants (b3a2, b2a2, e1a2) which translate to fusion proteins p210 and p190. The b3a2 and b2a2 transcripts for p210 were detected in all 30 CML patients (100%). Additionally, 2 out of the 30 patients (6.6%) simultaneously exhibited e1a2 transcripts coding for p190. In conclusion, the major BCR-ABL fusion transcript identified in Pakistani CML cases is the b3a2/b2a2 subtype producing p210 fusion protein. A smaller subset shows co-expression of the minor e1a2 variant encoding p190. These findings help characterize the prevalence of distinct BCR-ABL isoforms within the native CML patient population. Identifying transcript types is essential for monitoring disease progression and tailoring targeted therapies. Further studies with larger sample sizes are needed to explore the role of transcript variations in disease presentation and management.

Keywords: *CML, PCR, real time PCR, Fusion transcripts, TKI's.*

OP-31

Unveiling the Anticancer Potential of Antarctic Yeast Extracts against Ovarian Cancer Cells'

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ABSTRACT

Ovarian cancer is one of the leading causes of cancer-associated mortality in women worldwide. About 70-80% of patients are diagnosed at a highly inclusive stage of growth. As we know, Antarctica is a geographical polar region, and microorganisms over there have attracted attention as a useful source for novel therapeutics, including anticancer drugs. So, this review paper investigates the effects of citromycin, isolated from the Antarctic marine-derived fungus Sporothrix sp., on human ovarian cancer

cells. Other research agencies working on this are facing problems in storing this yeast as it thrives in extremely cold environments. Also, their growth rates are very slow, which limits their biomass yield. By using bioreactors and genetic engineering techniques, these problems can be solved. By critical analysis and narrative review of other research papers along with conceptual reasoning, this article will investigate how this Antarctic yeast affects patients with Ovarian cancer and how it can be obtained in larger quantities using specialized techniques. Citromycin inhibited the migration of human ovarian cancer cells and regulated the gene expression, highly impacting the symptoms of ovarian cancer positively. The symptoms were alleviated and patients recovered from their cancer in due time, due to this miracle fungus. Their yield was also increased using specialized procedures and bioreactors. Access to innovative cancer treatments could significantly increase survival rates and improve the quality of life. If production is scaled efficiently, Antarctic yeast-derived therapies might offer cost effective alternatives to expensive cancer drugs especially in low-income countries. Further research needs to be carried out into this field to make sure man makes the right use of Nature's gifts, just like this one.

Keywords: *Human ovarian cancer treatment, Antarctic yeast, Citromycin, Sporothrix sp.*

OP-32

Effect of Palmitate Treatment on Doxorubicin Response of Breast Cancer Cell Line

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ABSTRACT

Breast cancer is a multifactorial disease that impacts millions of women worldwide. Despite the wide range of available therapeutic choices, relapse and poor drug response are a few of the major challenges to successful control of the disease. Altered lipid metabolism has emerged to play a significant role in breast cancer induction, progression and metastasis. Few studies have addressed the effect of lipids uptake/synthesis on cancer treatment. Palmitate is the most common saturated fatty acid found in the diet or synthesized endogenously. The current study aimed to analyze the effect of palmitate on the drug response of breast cancer cells. MCF-7, a primary breast cancer cell line was selected for the current study. Na-palmitate conjugated with bovine serum albumin was used as an exogenous lipid source. Cells treated with and without Na-palmitate were subject to anti-breast cancer drug doxorubicin. MTT, a cell viability assay and apoptosis assay were performed to assess the drug response. Further gene expression analysis was performed to analyze the regulation of selected genes involved in apoptosis, lipid metabolism, proliferation and cell cycle regulation. Oil red O (ORO) staining and lipid extraction/quantification were performed to estimate the de novo lipid synthesis and

accumulation. Robust doxorubicin response in the presence of Na-Palmitate was further validated by significant upregulation of apoptosis, and cell cycle arrest genes along with downregulation of cell proliferation genes. In addition, lipid quantification and genes were differentially regulated in palmitate, followed by doxorubicin treatments. In conclusion, palmitate can be employed as a carrier molecule of doxorubicin to enhance its response in breast cancer for improved treatment.

Keywords: *Palmitate, Drug Response, Doxorubicin, Breast Cancer.*

OP-33

Harnessing the Therapeutic Potential of Incensole Acetate Terpene Nanoemulsion in Targeting Breast Cancer: A Translational Approach Toward Therapeutic Strategies

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ABSTRACT

The characteristics of phytocompounds have undergone extensive research in the medical and pharmaceutical sectors due to their extensive usage. To enhance cancer diagnostic and treatment criteria, novel bioactive compounds with increased efficacy are always needed. The primary goal of the study was to examine the therapeutic activity of terpene phytocompound that has been extracted as well as terpene-based nano emulsions against breast cancer biomarkers and WWP1 gene expression in cancer. The terpene compound incensole acetate was isolated from Catharanthus roseus and used for computational docking analysis. The binding potential and inhibitory effect of the identified terpene compound to target breast cancer receptors, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor2 (HER2) was assessed by using molecular docking, ADMET analysis and dynamic simulation approaches. For the assessment of in vitro antitumor activity, breast cancer cell lines were used to evaluate the cytotoxicity of essential oil, isolated terpene compound and terpene-based nano emulsion. The administration of terpene based nanoformulation reformed the biochemical, stress markers, and inflammatory markers and exhibited significant anticancer activity. The antioxidant enzymes, superoxide dismutase, glutathione reductase, and catalase were found to be significantly improved in terpene-treated groups as compared to DMBA cancer-induced animals. The anti-proliferative outcome was further assessed by a gene expression study of selected genes involved in breast cancer progression. The results of nanoemulsion treatment were found more significant on WWP1 expression levels. It was concluded that natural isolated terpene based nanoformulation used in this study an effective and promising formulation against DMBA-induced cancer in rat model. All

these results contribute to support these findings that terpene from the Catharanthus roseus plant have impending effects to prevent breast cancer inflammatory biomarkers, reduce level of oxidative stress enzymes and help in down regulation of cancer proliferation gene expression.

Keywords: *Breast cancer; nanoemulsion; cytokines; terpenes; phytocompounds; gene expression.*

OP-34

Evaluating Sotorasib and Adagrasib Against Docetaxel in Advanced Non-Small Cell Lung Cancer Treatment: A Systematic Review and Meta-Analysis

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ABSTRACT

This study aims to compare the efficacy and adverse event profiles of KRAS inhibitors, particularly sotorasib and adagrasib, with the traditional chemotherapy agent docetaxel in the treatment of Non-Small Cell Lung Cancer (NSCLC). We conducted a comprehensive analysis of clinical trials and studies involving sotorasib, adagrasib, and docetaxel. Key metrics such as overall response rate (ORR), progression-free survival (PFS), overall survival (OS), and adverse event rates were collated and compared.

In the meta-analysis, 12 studies were selected, including a total of 3,215 patients. Out of the total, 1,395 patients were treated with KRAS inhibitors, while 1,820 received docetaxel. The ORR for KRAS inhibitors was 36.11%, significantly higher than that of docetaxel (19.34%). Furthermore, the survival rate increased to 13.44 months for patients receiving KRAS inhibitors. Although treatment-related adverse events occurred in both cases, the severity of those events was lower with KRAS inhibitors. However, KRAS inhibitors were associated with higher rates of adverse gastrointestinal effects, such as diarrhea (58.13%) and vomiting (37.6%). In contrast, docetaxel caused more severe hematologic toxicities, including anemia (47.07%) and neutropenia (42.33%). This study underscores the importance of personalized medicine in NSCLC treatment. Sotorasib and adagrasib offer significant advantages for patients with specific genetic mutations, though their gastrointestinal side effects require careful management. Docetaxel, despite its broader applicability, is associated with notable hematologic adverse effects. The choice of treatment should be guided by the patient's genetic profile and ability to manage specific side effects, emphasizing the need for tailored therapeutic strategies in NSCLC.

Keywords: *Non-Small Cell Lung Cancer (NSCLC), KRAS inhibitors, Sotorasib, Adagrasib, Docetaxel, Efficacy, Personalized Medicine.*

OP-35

Immuno-Informatics-Based Prediction of Putative T-Cell Epitopes for Pancreatic Cancer Vaccine

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ABSTRACT

Pancreatic cancer is a highly lethal disease, ranking as the 12th most common cancer worldwide and a leading cause of mortality in affluent countries. It primarily results from DNA mutations that lead to uncontrolled cell division. The most prevalent type, adenocarcinoma, accounts for 85% of cases, while pancreatic endocrine tumors constitute about 5%. Key risk factors include smoking, genetic predisposition, diabetes, obesity, alcohol consumption, and physical inactivity. Conventional treatments such as surgical resection and chemotherapy have had limited success, with over 90% of patients succumbing to the disease. To develop a more effective approach, researchers have turned to in silico vaccine design using bioinformatics tools. This involves selecting antigenic proteins and epitopes that stimulate the immune system. Ten cytotoxic T lymphocyte (CTL) and ten helper T lymphocyte (HTL) epitopes were identified based on their antigenicity, stability, and immunogenicity. Proteins such as TNFRSF21, FGFR1, ITGA6, and others were chosen for their strong immune response potential. The selected epitopes underwent molecular docking with MHC-I and MHC-II receptors to ensure effective immune system activation. The vaccine construct was designed by linking CTL epitopes with the AYY linker, HTL epitopes with the GPGPG linker, and adding an EAAK linker at the end, followed by an HBD3 adjuvant to enhance immunogenicity. Structure validation, immune simulation, and in silico cloning confirmed the vaccine's efficacy. When tested in simulations, the vaccine produced antibodies within 20–30 days, with protection lasting up to 350 days. It also stimulates interleukins like IL-6, IL-10, and IL-18, which aid in eliminating cancer cells and promoting immune memory. This polyvalent vaccine construct offers a promising alternative to conventional treatments, providing long-term protection and an enhanced immune response against pancreatic cancer.

Keywords: *Pancreatic Cancer, Immuno-informatics, Vaccine, Molecular docking*.

OP-36

Advanced Molecular Diagnosis and Prevalence of Multi-Drug Resistance and Extensive Drug Resistance Tuberculosis with GeneXpert

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ABSTRACT

The increasing prevalence of Mycobacterium tuberculosis with multi-drug resistance (MDR-TB) and extensive drug resistance (XDR-TB) poses a significant challenge in healthcare, which demands a comprehensive understanding of its patterns and resistance profiles. The present study aims to diagnose Mycobacterium tuberculosis (MTB) and to determine the frequency of multidrug resistance (MDR-TB) and extensive drug resistance (XDR-TB) among the samples collected from patients of a tertiary care hospital. A total 594 sputum samples were collected from suspected MTB patients of age ranging from 20 to 65 years. The MTB-Rifampicin (RIF) resistance samples of suspected patients were detected by using the MTB/RIF Ultra assay on the GeneXpert system. The samples with MTB detected and RIF resistance were further tested using the MTB/XDR assay to determine resistance against different drugs used for the treatment of MTB. The results revealed the presence of MTB in 184 out of 594 samples. Of these 184 samples, 32 were identified as having MDR-TB (5.4%), and 13 of those 32 were further identified as XDR-TB (2.2%). Rifampicin-resistant cases were further tested against Isoniazid showed 86%, Fluoroquinolone 24%, Ethionamide 13, Kanamycin and Amikacin each 5%, and Capreomycin showed 3% resistance. In conclusion, the high incidence of drug-resistant strains, particularly the significant proportion with Rifampicin and Isoniazid resistance, highlights the challenges in managing tuberculosis infection. These findings emphasize the need for continuing research and public health campaigns to develop effective strategies for managing and treating drug-resistant tuberculosis strains.

Keywords: *Mycobacterium tuberculosis, Multi drug resistance (MDR-TB), Extensive drug resistance (XDR-TB), GeneXpert system, MTB/RIF Ultra assay, MTB/XDR assay.*

OP-37

Production and Characterization of Adenovirus-Based VP2 Viral Vector Vaccine Against Local Isolates Causing Infectious Bursal Disease in Chickens

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ABSTRACT

An infectious bursal disease (IBD) is a contagious, immunosuppressive disease suffered by young chickens. With over 1.5 million people employed, the poultry industry in Pakistan is vital to the economy, but is highly susceptible to infectious diseases, with higher mortality rates in chickens. In addition to being an important structural protein, VP2, which makes up the outer capsid of IBDV, is recognized by neutralizing antibodies, making it a potential target for vaccine production. Among different types of vaccines, viral vector vaccines exhibit better efficacy against IBDV. This is due to their ability to induce a strong and longlasting immune response. The purpose of this study is to produce and characterize the VP2-based viral vector vaccine that can provide comprehensive protection against both local strains of IBDV and those circulating in the field. For this purpose, Human embryonic kidney (HEK293) cells have been transfected and infected with a previously constructed adenoviral vector (pAdEasy1-CMV-VP2) in order to develop vaccine. VP2-based Adenoviral vector vaccine has been produced in HEK293T/F adherent and suspension cell lines, followed by its titration through TCID50 and qPCR assays. Immunization studies have been conducted in chickens immunized with VP2 virus vector vaccines and challenged with local isolates of IBDV. Virus neutralization assay using anti-sera produced against VP2 viral vector vaccine and antibody titer using ELISA confirm the effective immune stimulation in chickens, making it as a very good candidate for IBD vaccines.

Keywords: *Infectious Bursal Disease Virus (IBDV), VP2 protein, Virus neutralization assay, Poultry.*

OP-38

An In Vivo Experimental Validation of the Predicted T-Cell Epitopes for Human Respiratory Syncytial Virus (HRSV)

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ABSTRACT

Respiratory Syncytial Virus (RSV) is a major cause of lower respiratory tract infections (LRTIs) in young children and infants. In Pakistan annually, ~0.125 million children of age <5 years die because of LRTIs. Against RSV infections, only prophylactic treatment (Palivizumab) is in practice as there is no proper medication and licensed vaccine available so far. This study was designed to investigate potential vaccine candidates against Pakistani serotypes of RSV by reverse vaccinology approach. For this purpose, RSV-related most antigenic localized peptide sequences were identified from the

available genomic and proteomic databases. The most antigenic T-cell epitopes (3RSV-MHC-I: FSSKFWPYF and 6RSV-MHC-II: FWPYFTLIH) for RSV were predicted (from SH protein of hRSV) and synthesized. The host-pathogen interaction was investigated. In vivo experimental validation of the synthesized epitope peptides was done in rats (Sprague Dawley) and the immune response was investigated through Hematology (WBCs, LYM, NEUT), IFN-γ, Granzyme B and IgG specific assays. It was observed that the doses of Peptides along with IFA and/or CFA resulted in relatively lower levels of WBCs, LYM, NEUT, IFN, IgG and Granzyme-B as compared to the doses of Peptides alone. The synthetic peptide 3RSV-MHC-I induced significantly higher levels of WBCs, LYM, NEUT, IFN, IgG and Granzyme-B when administered as 1st booster dose without any adjuvant. The levels of IgG and Granzyme-B were relatively higher with 6RSV-MHC-II peptide (2.97 g/L, 613 pg/ml respectively) than with 3RSV-MHC-I peptide. The results indicated that both synthetic peptides could be effective vaccine candidates against hRSV. This study helps in understating the RSV pathobiology and leads to the discovery of new vaccine candidates against RSV.

Keywords: *RSV, Reverse Vaccinology, T-cell epitopes, Synthetic peptides*

OP-39

Prevalence of Thyroid Autoimmunity in HCV Patients Despite Normal Thyroid Function Tests in Pakistan

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ABSTRACT

Hepatitis C virus (HCV) infection is high in Pakistan. HCV primarily causes hepatic complications but also causes extra-hepatic manifestations like thyroid dysfunction (TD). TD secondary to interferon treatment is well known but the role of HCV per se in inducing thyroid autoimmunity (TAI) is not yet explored. This study was planned to know prevalence of thyroid peroxidase antibodies (TPO-Ab) in HCV patients presenting with normal thyroid function tests (TFT). The ELISA positive HCV patients with TFT within normal ranges were selected for this study. Serum thyroid related hormones (Free T4, T3 and TSH) were determined by radioimmunoassay techniques (RIA) and TPO-Ab by ELISA method using commercial kits. Serum TPO-Ab titer >20.0 IU/ml were considered positive. The mean (± SD) age of selected HCV patients (n=330; female 247, male 83) was 36.7 ± 11.1 year (age range: 15-65 years). Among them 79 (23.9%) patients were positive for serum TPO-Ab. Female HCV patients had higher incidence as compared to male patients (25.9% versus 16.9%) but difference was not significant (p=0.246). HCV patients with and without TAI were not different with respect to mean age (p=0.948),

presence of goiter (p=0.956) and mean thyroid hormones (both p>0.05). However, patients with positive TPO-Ab had slightly higher TSH as compared to negative TPO-Ab patients (2.0± 1.1 versus 1.8± 0.8 mIU/L) but the difference was not significant (p=0.271). More than 20% of local ELISA positive HCV patients regardless of age, presence of goiter and TFT levels had thyroid antibodies despite normal thyroid function tests.

OP-40

RT-LAMP: Advancing HCV Variant Detection Beyond Commercial RT-qPCR

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ABSTRACT

Effective Hepatitis C Virus (HCV) elimination requires improved diagnostic strategies, particularly in high-burden regions like Pakistan. Rapid and accurate detection is essential, yet commercially available RT-qPCR assays often suffer from limitations in sensitivity, specificity, cost, and accessibility. RT-qPCR remains time-consuming and requires skilled personnel, whereas Reverse Transcription-Loop Mediated Isothermal Amplification (RT-LAMP) presents a robust, cost-effective alternative with superior diagnostic efficiency. This study aimed to develop and optimize an RT-LAMP assay targeting both Variants of Interest (VOI) and Variants of Concern (VOC) of HCV, addressing gaps in existing commercial assays, particularly in resource-limited settings. Degenerate RT-LAMP primers were designed to target the 5' UTR of HCV, incorporating sequences from reference genomes and emerging genotypes, including those prevalent in Pakistan. Critical assay parameters, including reaction time, volume, primer concentration, and temperature, were systematically optimized. Validation using clinical HCV samples demonstrated that the RT-LAMP assay achieved a detection limit of 131 copies, surpassing commercial RT-qPCR by correctly identifying 71% of false negatives. The assay exhibited 100% sensitivity and specificity, compared to RT-qPCR, which showed 82% sensitivity. Additionally, the RT-LAMP assay required only 35 minutes for processing, making it highly suitable for large-scale diagnostics. BLAST analysis of RT-LAMP primers against HCV reference genomes, Pakistani isolates, and emerging variants yielded E-values between 1e-06 and 6e-05, with no significant cross-reactivity to related Flaviviridae members such as Chikungunya, Dengue, and SARS-CoV-2. The newly developed RT-LAMP assay represents a major advancement in HCV diagnostics, providing a rapid, highly sensitive, and specific tool for improving detection, prevention, and control strategies, particularly in low-resource settings.

Keywords: *Nucleic Acid Amplification Tests, Point of Care testing, Isothermal amplification, RNA viruses, and high mutation rates.*

OP-41

Diagnostic Potential of GFAP, TNF- α and IL-6 in Stroke Subtypes and Severity

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ABSTRACT

Acute stroke is a prime neurological emergency needing immediate diagnosis and interference to minimize brain damage and enhance recovery. Distinguishing IS from ICH is vital due to separate treatment plans. As CT and MRI are accustomed diagnostic tests, their limitations require interrelated applications of various tools. This research focused to assess serum Glial Fibrillary Acidic Protein (GFAP), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-6 (IL-6) levels during first 4.5 hours of symptom onset to distinguish IS from ICH and evaluate their relation with disease intensity, calculated by the National Institutes of Health Stroke Scale (NIHSS). This Cross-sectional study included 176 participants (88 stroke patients and 88 healthy controls) recruited from Lahore General Hospital, Lahore. Serum GFAP, IL-6, and TNF-α levels were measured and analyzed using the Mann-Whitney U and Kruskal-Wallis tests. Correlations with NIHSS were assessed using Spearman's rank correlation, and diagnostic accuracy was evaluated via Receiver Operating Characteristics (ROC) curve analysis. Median serum levels of GFAP, IL-6, and TNF-α were significantly higher in stroke patients compared to healthy controls (GFAP: 1.90 vs. 0.72, IL-6: 21.2 vs. 6.48, TNF-α: 129.4 vs. 78.7; all $p < 0.0001$). GFAP levels were significantly higher in ICH compared to IS patients (1.33 vs. 0.81, $p < 0.05$). NIHSS scores correlated strongly with GFAP ($r = 0.81$, $p < 0.001$) and moderately with IL-6 and TNF-α levels. ROC analysis showed excellent diagnostic accuracy for distinguishing stroke from controls (GFAP AUC: 0.907, IL-6 AUC: 0.901, TNF-α AUC: 0.891), with a combined model providing the highest accuracy (AUC: 0.965). For differentiating ICH from IS, GFAP was the most effective stand-alone biomarker (AUC: 0.816), while the combined model achieved the highest diagnostic performance (AUC: 0.858). Through our results, we may suggest that serum GFAP, IL-6, and TNF-α levels effectively distinguish IS from ICH and correlate with stroke severity. GFAP emerged as the most reliable individual biomarker, while the combined model demonstrated superior diagnostic accuracy. These biomarkers show promise for rapid diagnosis and management of acute stroke, particularly in resource-limited settings where imaging modalities are unavailable. Standardized thresholds and further large-scale validation are needed for clinical integration.

Keywords: *Stroke, IS-Ischemic Stroke, ICH- Intracranial Hemorrhage, GFAP-Glial Fibrillary Acidic Protein, TNF- α - Tumor Necrosis Factor-alpha, IL-6 - Interleukin-6, NIHSS- National Institutes of Health Stroke Scale, ROC- Receiver Operating Characteristics curve analysis.*

OP-42

Biofilm Forming Potential and Occurrence of Hospital Associated Marker (IS16 Gene) Among *Enterococcus Faecalis*

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ABSTRACT

Bacteria in soil, especially *Enterococcus faecalis,* plays many crucial roles such as maintaining soil structure and supplying nutrients to plants. In soil, biofilms formed on organic particles play a major role in the decomposition of litter, whereas those formed in the rhizosphere help promote plant growth and protect against disease. In addition, quorum-sensing signals within biofilms lead to the up- and downregulation of gene expression, allowing adaptation to various environmental organic contaminants. Soils have a natural input of pathogenic microorganisms from different sources such as sewage, recipient water and fertilizers of animal origin, which may pose a threat to the community. Enterococci are characterized as Gram-positive, catalase-negative, non-spore forming, and aero-tolerant fermentative organisms that form the second largest group of bacteria studied regarding microbial source tracking. Despite being a member of normal human intestinal flora, they are not regarded any more as GRAS (Generally Recognized as Safe) organisms. We have examined the most potential part of our ecosystem, i.e. soil, which is a very complex mixture of air, water, dead organic matter, and various types of living organisms responsible for its broad metabolic capacity. We examined the potential of biofilm formation and prevalence of hospital associated marker (IS16 gene) along with antibiotic resistance and virulent factors associated with enterococci (n=372) from bulk soil samples (n=500) of Karachi, Pakistan. BOX and RAPD PCR were used to determine genetic relatedness among different species. Majority of the virulence factors were associated with E. faecalis followed by E. faecium. Varying degrees of resistance against different antibiotics were noted. In conclusion, Presence of multiple antibiotic resistant, virulent and hospital associated enterococci in bulk soil represents a potential source for further dissemination to human and animals.

Keywords: *Biofilm, Enterococcus faecalis, Soil, Hospital associated marker (IS16 gene).*

OP-43

High Frequency and Molecular Characterization of ESBLs and Carbapenemases producing Gram-negative Bacterial Isolates from Burn Patients

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ABSTRACT

The prevalence of multi-drug-resistant bacterial pathogens isolated from burn patients increased dramatically in the recent decade, demonstrating a severe health issue in burn units worldwide. This study was conducted to provide a framework for the rapid profiling of antimicrobial resistance genes in MDRs bacterial pathogens. Cephalosporins and carbapenems resistant Gram-negative isolates from burn patients were analyzed to determine the occurrence of antimicrobial resistance. CLSI guidelines 2017 were followed for the antimicrobial susceptibility testing (AST) and ESBLs/carbapenemase screening. Molecular profiling of ESBLs was accomplished by multiplex PCRs bla_{CTXM-1}/bla_{CTXM-3} and bla_{OXA-1}/bla_{TEM}/bla_{SHV}. Molecular detection of carbapenemases was done by the multiple bla_{OXA48}-bla_{KPC}-bla_{NDM}-bla_{VIM} and single bla_{IMP-1} PCRs. Multiple antibiotic resistance values (MAR index) of 400 MDRs, ranged between 0.59-1.00. Molecular profiling of ESBLs by multiplex PCRs bla_{CTXM-1}/bla_{CTXM-3} and bla_{OXA-1}/bla_{TEM}/bla_{SHV} confirmed 79 (19.8%) bla_{CTXM-1}, 58 (14.5%) bla_{CTXM-3}, 62 (15.5%) bla_{TEM}, 31 (7.8%) bla_{OXA-1}, and 24 (6.0%) bla_{SHV} positive isolates. Multiple genotypes including CTXM1-TEM and OXA1-TEM A. baumannii and OXA1-TEM-SHV P. aeruginosa caused MAR index of 1 and MICs of 32µg/ml for cefotaxime and 64µg/ml for ceftazidime. Genotyping of carbapenemases by conventional bla_{IMP-1} and multiplex bla_{OXA-48}/bla_{KPC}/bla_{NDM}/bla_{VIM} PCRs identified 53 (13.3%) bla_{IMP-1}, 45 (11.3%) bla_{VIM}, 35 (8.8%) bla_{OXA-48}, 29 (7.3%) bla_{KPC}, and 14 (3.5%) bla_{NDM} positive isolates. Multiple carbapenemases producing genotypes including OXA48-VIM P. aeruginosa, OXA48-KPC K. pneumoniae, KPC-VIM P. mirabilis, and OXA48-KPC-VIM Enterobacter cloacae were also detected with 0.76-0.82 MAR and ≤ 32µg/ml MICs for imipenem and meropenem. ESBLs and carbapenemases producing Pseudomonas spp., and Enterobacteriaceae are becoming a real challenge for the survival of burn patients. The higher rates of bla_{TEM}, bla_{OXA}, bla_{SHV}, bla_{VIM}, and bla_{NDM} genes confirm the need to improve the management of burn patients to prevent post-burn infections.

Keywords: *Burns, Carbapenemases, Enterobacteriaceae, ESBLs, blaOXA48, Pseudomonas aeruginosa, blaVIM.*

OP-44

Whole Genome Sequencing-Based Analysis of Antimicrobial Resistance and Virulence Factors in *Salmonella gallinarum* Isolated from Poultry

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ABSTRACT

Salmonella gallinarum is a host-adapted pathogen that causes fowl typhoid disease in poultry. Despite implementing sanitation practices and prophylactic measures on farms, it frequently causes outbreaks in developing countries, including Pakistan. This study aimed to identify the key genomic features and virulence potential of *Salmonella gallinarum* SG-1 isolated from a fowl typhoid outbreak in poultry. The complete genome of *Salmonella gallinarum* SG-1 was sequenced using Illumina sequencing technology and annotation was performed using the Prokaryotic Genome Annotation Pipelines (PGAP). The genomic features of isolate were analyzed using the online tool CGview server and antibiotic resistance genes, virulence factors and prophage loci were identified using various online tools. The complete circular chromosome of *Salmonella gallinarum* SG-1 isolate is 4624151 bp long with a GC content of 52.1% and contains a total of 4471 genes. The isolate showed more than 98% sequence similarity with reference genome (Accessioon no. CP088134.1) as analyzed by Average Nucleotide Identity (ANI) tool. The results revealed the various antibiotic resistance genes including those for multidrug efflux pump (acrB), modifying bacterial surface (pmrA, pmrb) and fluoroquinolones resistance (mutation in p.D87Y, parC, parE, gyrA, gyrB), as well as several 16S-rrsD genes and aminoglycoside resistance aac(6')-laa analyzed by Resfinder-4.6.0. Moreover, the genome contains the genes encoding for Salmonella pathogenicity islands SPI-1, SPI-2, SPI-3, SPI-5, SPI-10, SPI-12, SPI-13, SPI-14, CS3P1 and CS54 which contribute to evading the host defense and causing infection. The findings of this study demonstrate the genetic basis for virulence factors, prophage genes and multidrug resistance in *Salmonella gallinarum* SG-1. The information obtained highlights the importance of continual surveillance and understanding this genetic data may help in developing new interventions or strategies to control *Salmonella gallinarum* in poultry.

Keywords: *Illumina sequencing, Fowl typhoid, antibiotic resistance, Salmonella gallinarum.*

OP-45

Novel Antibacterial Activity of Nitro Group in Small-Molecule Sulphonamides against Antibiotic Resistant Uropathogenic *E. coli*

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ABSTRACT

Uropathogenic Escherichia coli (UPEC) causes urinary tract infections (UTIs) in approximately 150 million people worldwide each year. It is estimated that 40% of women will experience at least one UTI during their lifetime. UTIs are caused by a wide range of pathogens, including Gramnegative and Gram-positive bacteria presenting a significant public health challenge. E. coli is the predominant causative agent of both uncomplicated and complicated UTIs. In an era of increasing bacterial resistance to antimicrobial agents, coupled with a high prevalence of multidrug-resistant (MDR) strains in community and hospital-acquired infections, it is essential to re-evaluate existing antimicrobial agents. In this context, the use of Sulfamethoxazole derivatives appears to be a reasonable approach.

This study aimed to evaluate the activity of newly synthesized fluorinated sulfonamides against common Uropathogens, comparing them to the widely used Sulfamethoxazole and other antimicrobial agents routinely used for UTI treatment. The bactericidal activity of our sulfonamide compounds against resistant UPEC strains was assessed by determining the Minimum Inhibitory Concentration (MIC) and comparing their effectiveness to standard antibiotics. We hypothesized that the synthetically prepared fluorinated sulfonamides would exhibit significant bactericidal activity against resistant UPEC strains.

Among the compounds tested, Compound 4g demonstrated lower MIC values compared to Sulfamethoxazole when tested against Gram-negative bacteria, including clinical MDR *E. coli* strains. The MIC values for Compound 4g ranged from 62.5–125 µg/mL for Uropathogenic *E. coli* strains, while the MIC for Sulfamethoxazole exceeded 500 µg/mL for the same resistant strains. To identify the antibacterial component of the active compound, we performed activity assays with and without the presence of nitro groups. Our results showed that the nitro group was crucial for the compound's activity, as the removal of the nitro group from Compound 4g led to a complete loss of antibacterial activity. Therefore, our study confirmed that the antibacterial properties of Compound 4g are attributed to the nitro group rather than the sulfonamide moiety.

Keywords: *Multi-drug-resistant Bacteria, Sulfonamides, Uropathogenic E.coli, Minimum inhibitory Concentration (MIC).*

OP-46

In Vitro Efficacy of Meropenem-Vaborbactam Against Carbapenem-Resistant *Pseudomonas aeruginosa*

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ABSTRACT

The Gram-negative bacterium Pseudomonas aeruginosa is a common cause of opportunistic infections, especially in hospital settings, and poses serious therapeutic difficulties because of its high level of antibiotic resistance. Meropenem-vaborbactam (MEV) is a novel β-lactam/β-lactamase inhibitors combination drug that exhibits activity against carbapenem-resistant P. aeruginosa. Therefore, this study focuses on the effectiveness of MEV against carbapenem-resistant P. aeruginosa isolates. A total of 280 clinical samples were collected from different wards and analyzed in a cross-sectional study carried out at the pathology department of a hospital in Lahore. The isolates were initially identified through culture characteristics on MacConkey agar, later confirmed by cytochrome oxidase assay. The antimicrobial susceptibility of organisms against different antibiotics was evaluated using the Kirby-Bauer disc diffusion method by following Clinical and Laboratory Standards Institute (CLSI). After that, minimum inhibitory concentration (MIC) of MEV against carbapenem resistant P. aeruginosa were done by using Vitek-2 compact system. Out of 280 isolates of P. aeruginosa, only 84 (30%) were found to be carbapenem-resistant, with the highest number isolated from skin and soft tissue infections (SSTIs) (n=47), followed by tracheal secretions (n=22) and urine samples (n=15). Maximum resistance was observed against cephalosporins 100%, followed by piperacillin-tazobactam 89%, gentamicin 81%, and amikacin 76%. Interestingly, 83% of isolates showed susceptibility to colistin, indicating its potential as a therapeutic option. MICs ranged from 2 to 64 µg/mL, with many isolates 72 (86%) exhibiting resistance to meropenem-vaborbactam at an MIC value of >64 µg/mL, while only 12 (14%) isolates were susceptible at an MIC of 8 µg/mL. The limited efficacy of meropenem-vaborbactam against carbapenem-resistance P. aeruginosa is alarming but its effectiveness can be considered in treating MDR organisms. Continuous monitoring and further research in the development of alternative treatment options for this organism is warranted.

Keywords: *Pseudomonas aeruginosa, Meropenem-Vaborbactam (MEV), Carbapenem resistant, Antimicrobial susceptibility, Piperacillin-Tazobactam, Minimum inhibitory concentration (MIC).*

OP-47

Assessment of Anticancer and Antimicrobial Potential of Bioactive Metabolites and Optimization of Culture Conditions of *Pseudomonas Aurantiaca* Pb-St2 For High Yields

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ABSTRACT

The following study aimed to characterize the biological potential of the purified compounds of Pseudomonas aurantiaca PB-St2. Optimization of temperature and incubation time of 32 ºC and 72 h yielded the highest crude extract weight and optical density of bacterial culture. HPLC analysis of the crude metabolite extract (purified using gravitational column chromatography) showed three fractions named PC1, PC2, and PC3. HPLC-purified fractions were subjected to LC-MS/MS analysis and the data was compared using reference library. Fraction PC1 was identified as mupirocin, PC2 as phenazine-1-carboxylic acid (PCA), and PC3 as the mixture of three compounds including pyoluteorin, PCA and 2-hydroxyphenazine (2-OH-phz). Fungicidal potential of the purified compounds was assessed against phytopathogens including Fusarium equiseti, Fusarium incarnatum, Alternaria alternata, and Colletotrichum falcatum. Fraction PC3 showed the highest fungicidal activity of ~89%, whereas the least antifungal activity (~27%) was noted for mupirocin. Antibacterial activity of the purified compounds against Gram-positive pathogen Bacillus cereus, and Gram-negative pathogens Pseudomonas aeruginosa, Salmonella enterica, and Klebsiella oxytoca were also assessed. Fraction PC3 demonstrated the highest antibacterial activity against B. cereus and P. aeruginosa, showing 1.8 cm and 0.9 cm zones of inhibition, respectively. Against Klebsiella oxytoca and Salmonella enterica, the antibacterial activity of PB-St2 crude extract was slightly higher than the fraction PC3. The fraction PC3 also demonstrated the highest IC_{50} against HepG-2 and SF767 cancer cell lines at 25 µg and 20 µg concentrations, respectively. The multifaceted attributes of *P. aurantiaca* PB-St2 make it an ideal candidate for agricultural and pharmacological applications.

Keywords: *Anticancer, Antimicrobial, Bioactive metabolites, Chromatography, P. aurantiaca.*

OP-48

Morphological Properties of Laser Irradiated Biomaterials

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ABSTRACT

On heating di-calcium phosphate dehydrate $CaHPO_4.2H_2O$ to 750℃, the β-$Ca_2P_2O_7$ is obtained. The materials were then pressed at 4 tons for 5minutes for preparation of pallets. The pallets were then driedat 120oC in a vacuum Chamber for 4 hours. The materials were sintered in a muffle furnace at 450ºC for 24 hours, this resulted to grey colour γ-$Ca_2P_2O_7$ phase. Further sintering at 750 ºC for 24

hours resulted in tetragonal beta $Ca_2P_2O_7$ phase with lattice constants to obtain a=b=6.684A and c=24.144A. The pallets were prepared from Di-Calcium Phosphate Di-Hydrate triclinic $2CaHPO_4.2H_2O$ powder was used to obtain three different phases Gamma, Beta , Alpha. These three different phases were obtained at 450ºC,750ºC and 1050 ºC respectively. The pallets having mean thickness of 0.12 cm were characterized by monochromated x-Ray Diffraction technique, high resolution optical microscopy. Before sintering micro-cracks were frequently seen on the palleted surfaces but then cracks disappeared after sintering at 750C.Grey coloured gamma phase was found to be harder than the white-coloured Beta Phase. SEM were also recorded for 450ºC and 75ºC pallets sintered for different periods and laser irradiation with Nd:YAG laser with some special specifications.

Keywords: *sintering, morphological, grey,calcium phospoate,* $CaHPO_4$.

OP-49

Polygenic Risk Score (PRS) Analysis of Genetic Variants in A Pediatric Pakistani Population with Ventricular Septal Defects (VSDS)

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ABSTRACT

Ventricular septal defects (VSDs), a congenital cardiac disease is the major abnormality of the heart that contributes to 40% of neonatal mortalities in the first month of childbirth. VSDs is a complex disease that is the result of interaction of various genetic determinants in regulators, transcription factors and enzymes including GATA4, SMAD7, VEGF, MTRR and ISL1.The understanding of genetic variations that contribute to VSDs is still underreported in the Pakistani population. Genotyping of seven polymorphisms was performed on 100 pediatric subjects (50 VSDs patients and 50 controls) by using Tetra ARMS-PCR and PCR–RFLP methodology. The single and polygenic variant analysis was conducted to identify the risk variants. The results of the analysis showed that MAF of all selected variants was significantly associated with VSDs. The GATA4 rs4841587 [OR 0.40 (95% CI 0.15–1.01)], SMAD7 rs3736242 [OR 0.26 (95% CI 0.08–0.81)], SMAD7 rs16950113 [OR 0.48 (95% CI 0.26–0.88)], VEGF rs699947 [OR 0.89 (95% CI 0.51–1.55)] variants showed significant protective impact, whereas GATA4 rs104894073 [OR 1.19 (95% CI 0.67–2.12)], MTRR rs1532268 [OR 1.00 (95% CI 0.57–1.75)], ISL1 rs6867206 [OR 1.39 (95% CI 0.76–2.55)] variants showed association with increased risk of VSD. Genetic contrast analysis demonstrated that the GATA4 rs104894073, VEGF rs699947 and MTRR rs1532268 increased risk of VSDs in the dominant model and the heterozygous genotype in the co-dominant model. In contrast, polygenic risk score does not suggest conclusive results. Our findings especially for GATA4 rs104894073, VEGF rs699947 and MTRR rs1532268 variants need to be validated in future studies. Also, a more effective model of PRS

should be developed that has more significant predictive power especially for the candidate SNP analysis.

Keywords: *Ventricular septal defects (VSDs), Polygenic risk score (PRS), Genetic models, Pediatric population.*

OP-50

Identification of Novel Missense Pathogenic Variant in FGFR3 Gene Causing Achondroplasia (ACH) In Pakistani Patients

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ABSTRACT

Achondroplasia (ACH; OMIM# 100800) is a genetic disease, an autosomal dominant skeletal dysplasia with associated phenotype of disproponate short-limbed dwarfism. The only causative gene responsible for disorder is Fibroblast growth factor receptor 3 (FGFR3, OMIM# 134934), located on chromosome 4p16.3 with 19 exons. Along with this ,FGFR3 also described for certain other genetic abnormalities like lacrimoauriculo-dento-digital (LADD) syndrome, Muenke syndrome, Crouzon syndrome with acanthosis nigricans, epidermal nevi , SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans), thanatophoric dysplasia, camptodactyly, tall stature, hearing loss syndrome (CATSHL syndrome). The current study designed for investigation of a Pakistani family with short stature disproponate skeletal dysplasia without cousin marriage history. Affected girl was 2.5years old born from short height parents indicated severe clinical and radiological phenotypes including severe short-limbed short stature with marked ossification abnormalities and significant delay in speech. Our affected members reported with very short upper and lower limbs with large head circumference which differentiated from other disorders. Genomic DNA was extracted from the proband and proceeded for whole-exome sequencing. A novel missense mutation (c.1144 G>A, p.Gly382Arg) in FGFR3 gene was identified and confirmed its cosegregation within family by Sanger sequencing which is predicted to cause damaging effects and likely to lead nonsense nonsense-mediated abnormality. This confirms the fact that pathogenic variant in FGFR3 is the major cause of achondroplasia and may be for other related phenotypes. The novel missense variant in FGFR3 gene is responsible for the unique phenotype of skeletal dysplasia (achondroplasia)

Keywords: *Achondroplasia, FGFR3, Muenke syndrome, Megaloencephalopathy, skeletal dysplasia.*

OP-51

Whole Genome Sequencing of Lumpy Skin Disease Virus (LSDV) in Indigenous Breeds of Cattles: A Step Forward for the Prevention and Treatment

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ABSTRACT

Lumpy Skin Disease (LSD) is a viral disease that affects cattle, causing significant economic losses and animal suffering. The disease is characterized by the appearance of nodules or lumps on the skin, typically accompanied by fever, lethargy, and loss of appetite. LSD is caused by the Lumpy Skin Disease virus (LSDV), a member of the Poxviridae family. The disease is transmitted through insect vectors, such as mosquitoes and ticks, as well as through direct contact with infected animals. Mechanical transmission by vectors is the prime route of spreading disease. The thick Amblyomma spp., Rhipicephalus sp and Hyalomma sp have been reported as mechanical vectors and reservoirs of viruses. The bitting flies (*Stomoxy calictrans*) and mosquitoes (e.g. Culex mirificens and Aedes aegypti), are also involved in the mechanical transmission of disease. The whole genome sequencing (WGS) of LSDV can be a powerful tool for understanding the molecular mechanisms of LSD. WGS can help identify genetic markers associated with disease susceptibility. By applying this molecular approach, it will become convenient to identify genetic markers associated with disease susceptibility, develop diagnostic tests and vaccines, improve disease management and control strategies, and enhance our understanding of the molecular mechanisms underlying LSD. This research has significant implications for the development of effective control measures and treatment strategies for LSD, ultimately reducing the economic and animal welfare impacts of the disease.

Keywords: *LSDV, Whole genome sequencing, Vector Borne Disease.*

OP-52

A Molecular Genetic Association Study of Prothrombin (F2) Gene rs1799963 G/A Polymorphism and Its Role in Atherosclerosis

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ABSTRACT

Atherosclerosis is a chronic inflammatory disease in which lipids, calcium, and fibrous tissues accumulate in arteries. It results in information on plaques. These plaques affect the blood flow and lead to fatal cardiovascular events, including stroke, peripheral artery disease (PAD), and coronary artery disease (CAD). Atherosclerosis is the leading reason for morbidity and mortality all over the world. This study examines the genetic association of F2 rs1799963 polymorphism with the development and progression of atherosclerosis in samples (n=122) collected from the Sargodha, a city of Punjab, Pakistan. 122 subjects were recruited for sampling, including 82 diseased with atherosclerosis and 40 controls. 5 ml of blood was extracted from each subject with the help of expert phlebotomists. All the patients were confirmed atherosclerotic. The DNA was extracted from these blood samples manually using PCI extraction method and amplified by using TRI-ARMA PCR. Statistical analysis was performed by using IBM SPSS 26.0 version. The genotypic distribution was GG genotype 81%, GA 16% and AA 03%. The prevalence of G allele was higher in diseased as compared to the control (92% vs 84%). GG genotype frequency was observed higher in the diseased, referring to the G allele may increase the risk. The findings also show the potential role of genetic factors in the pathogenesis of atherosclerosis and emphasize the importance of genetic screening in atrisk populations. Further research is needed to clarify the mechanism by which this polymorphism plays its role in potential therapeutic interventions.

Keywords: *Atherosclerosis, F2 rs1799963 Polymorphism, Genetic Association, Prothrombin Gene, Cardiovascular Disease.*

OP-53

Whole Genome Amplicon Sequencing of Dengue and SARS-CoV-2 Viral Genomes Using Oxford Nanopore Technologies

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ABSTRACT

Viruses are major contributors to human diseases, including pandemics. The accurate identification of viral strains is essential for effective surveillance and management strategies. Whole genome sequencing (WGS) is a powerful tool for strain characterization, but sequencing uncultured viruses directly from patient samples remains a significant challenge. Whole genome amplicon sequencing offers a viable alternative for sequencing viral genomes from uncultured samples. Oxford Nanopore Technologies (ONT) enables long-amplicon sequencing, providing advantages over conventional sequencing platforms. Recent improvements in base-calling algorithms have enhanced data quality,

increasing sequencing accuracy. In this presentation, I will discuss key findings from the whole genome amplicon sequencing of SARS-CoV-2 and dengue virus samples from Pakistan, sequenced using the GridION platform. The results highlight the potential of ONT for rapid and cost-effective viral genome sequencing in clinical and epidemiological settings.

Keywords: Amplicons, long read sequencing, ONT, Adaptive Sampling, Clinical samples.

OP-54

Expression of MoSIGIII Trigger Unprecedented Enhancement in Sugar Contents in Transgenic Sugarcane

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ABSTRACT

Sucrose is essential human sustenance and the feedstock for over fifty percent of the world's fuel ethanol production. Primarily, it is extracted from sugarcane and beet. Several years have elapsed since efforts via conventional and molecular breeding to increase the concentration of conserved sugar in elite sugarcane cultivars. In recent times, bacterial isomerase enzyme genes have been efficiently cloned. These enzymes modify sucrose into carbohydrates that are palatable by humans but not assimilated by plants; additionally, they offer nutritional advantages in contrast to sucrose. Our hypothesis posited that plants could obtain beneficial carbohydrates and surpass the sugar yield ceiling by leveraging an appropriate expression pattern of sucrose isomerase (SI) under different promoter combinations. By transferring an SI gene developed for vacuolar compartmentalization, sugarcane lines exhibited substantial increases in the total quantity of stored sugar. Without a decrease in stored sucrose concentration, the high-value sugar isomaltulose accumulated in storage tissues, substantially enhancing the total sugar concentrations in harvested juice. Additionally, the lines that accumulated sugar more rapidly demonstrated enhanced sucrose transport, photosynthesis, and sink strength. This unprecedented increase in the concentration of sugar stored in leaf as well as in stalks delivers a fresh outlook on the interplay between sources and sinks in plants and holds a significant promise to ensure stable sugar production in sugarcane.

Keywords: *Sugar contents, Sucrose, Isomerase, Isomaltulose, Sugarcane*

OP-55

Biocontrol of Fusarium wilt in Bell pepper using Rhizospheric Bacillus strains with Multifunctional Plant Growth-Promoting Traits

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ABSTRACT

Fusarium wilt of bell pepper caused by Fusarium oxysporum f.sp. capsici is a devastating disease that significantly impacts crop quality and yield. Unlike pesticides, which repeatedly introduced environmental pollution and generated resistant pathogens, biocontrol agents boosted disease repression without damaging the environment. This study was focused on the management of disease in bell pepper by biological control with plant growth promoting Rhizospheric bacterial isolates. Simultaneously, we investigated the beneficial effect of bacterial isolates on the growth attributes of bell pepper plants. A virulent isolate of *F. oxysporum* f.sp. capsici was obtained from field-grown hot pepper plants with wilt symptoms. The identification was confirmed by morphological characterization and microscopy of isolated pathogen. Bell pepper plants were raised in sterilized peat moss potting mix drenched with aqueous suspension of Plant Growth Promoting Rhizobacteria strains. After twenty days of emergence, the wilt pathogen was added in the plant growth medium using soil drenching technique. That is the experimental setup along with bacterial suspension and fungal inoculum preparation. After one month of incubation disease index and plant growth traits were noticed and recorded. These bacteria, Bacillus megatherium strain Z-06 and Bacillus simplex strain Z-09 significantly suppressed fusarium wilt disease. Additionally, both bacterial strains significantly increased growth parameters such as plant height, root length and plant biomass compared to non-treated control plants. Enzymes involved in phenylpropanoid pathways display increased activities in plants raised in the presence of B. megatherium strain Z-06 and B. simplex Z-strain 09 compared to pathogen control and non-treated control plants. To perceive the mechanism behind disease suppression and plant growth promotion, LCMS (liquid chromatography-mass spectrometry) system was utilized to perform HPLC-ESI-QQQ-MS/MS (high-performance liquid chromatography-electrospray ionization-triple quadrupole-mass spectrometry) analysis. The onset of Fusarium wilt disease significantly reduces the production of many primary and secondary metabolites inside bell pepper belonging to different metabolic pathways. Whereas the presence of beneficial bacterial microbes upgraded the production of metabolites comparable in treated plants that were comparable to non-treated control plants. This study further highlights the significance of Bacillus megatherium and Bacillus simplex strains in disease suppression and plant growth promotion through an eco-friendly approach.

Keywords: *Biocontrol, Fusarium wilt, HPLC-ESI-QQQ-MS/MS, LCMS.*

OP-56

Isolation and Characterization of Microbial Communities in The Root of Cicer Arietinum (Chickpeas)

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ABSTRACT

The aim of the present study was to isolation and characterization of microbial communities existing in the root nodules of chickpea plants, with a specific focus on the isolation of Agrobacterium species. The effective success of these objectives delivers appreciated insights into the diversity and potential functional characters of microbial communities associated with root nodules of chickpeas, specifically highlighting the existence of Agrobacterium as a significant component. The isolates from the root nodules were grown on LB agar media. Subsequently, the identification of Agrobacterium was carried out using various tests, including the antibiotic resistance test, gram-staining, microscopy, and pathogenicity test. Once the presence of Agrobacterium was confirmed, knockouts were generated by removing the tumor-causing genes from the Ti plasmid (tumor-inducing), resulting in the creation of Agrobacterium mutants. The mutants were then subjected to sequencing analysis.

OP-57

Genomic Insights into Phosphine Resistance in Trogoderma granarium through High-Throughput Sequencing.

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ABSTRACT

Phosphine is a widely utilized insecticide to combat *Trogoderma granarium*, one of the most destructive quarantine pests. However, the alarming rise in phosphine resistance poses a significant global challenge. This study explores the genetic underpinnings of resistance by performing a transcriptomic analysis of phosphine-treated and untreated strains of T. granarium. Six strains were assessed for susceptibility, revealing one susceptible strain and five with varying resistance levels based on LC_{50} values. Transcriptome assembly using Trinity (v2.10.0) produced over 110 million bases

and 194,926 genes. Differential expression analysis identified 19 key genes (p-value < 0.05), with nine upregulated and 10 downregulated in resistant strains. These genes are involved in essential biological processes, including muscular stability, insecticide detoxification, protein synthesis, ribosome integrity, and cellular energy regulation. These findings provide critical insights into the molecular mechanisms of phosphine resistance, paving the way for effective pest management strategies.

Keywords: *Phosphine; Stored grain pest; Transcriptome; Insecticide resistance; DE genes.*

OP-58

Genotoxicity Induced by Artificial Food Colors and Food Preservatives in Garlic (*Allium sativum l.*) Root Tips Cells

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ABSTRACT

Food additives such as artificial colors and preservatives are mutagenic and have been found to induce DNA damage in plant cells. Certain synthetic food additives have been found to induce DNA damage in plant cells, primarily in the form of mutations and chromosomal abnormalities. This research aimed to assess the genotoxic potential of two commonly used food colors (Allura red and tartrazine) and two preservatives (boric acid and sodium benzoate) on the root cells of garlic (Allium sativum L.) Garlic roots were treated with various concentrations of food colors (0.1, 01, and 10 g/L) and food preservatives (1, 2, and 4 g/L) for 24 h. Root tip cells were observed under the microscope to check for chromosomal abnormalities. The mitotic index observed a maximum of 11.32% at a 10 g/L concentration of Allura red and 19.88% for tartrazine at a 10 g/L concentration. The mitotic index for boric acid and sodium benzoate was at a maximum (9.80 and 10.95%, respectively) at a concentration of 4 g/L each. The abnormality index recorded for Allura red was 3.01%) and 2.98% for tartrazine at 10 g/L concentration. The abnormality index for boric acid was highest (5.88 %) at a concentration of 4 g/L. The abnormality index for sodium benzoate was maximum at 5.17% at 4 g/L. Overall, genotoxicity increased with the increasing concentration of food additives.

OP-59

Stem Cell-Based α-Terpineol-Loaded Hydrogels as Advanced Temporal Wound Dressings

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ABSTRACT

Burn wounds have significant clinical challenges due to severe tissue damage, delayed healing, and a high risk of infection. Traditional treatments often fail to promote complete tissue regeneration, necessitating advanced therapeutic strategies. Tissue engineering, particularly stem cell therapy and synthetic skin substitutes, offers a promising solution by enhancing complete wound regeneration. Mesenchymal stem cells (MSCs) play a crucial role in regulating inflammation, angiogenesis, and tissue repair. Moreover, hydrogels serve as semi-synthetic scaffolds that optimize stem cell therapy by providing a supportive microenvironment and improving cell retention. Therefore, this study evaluated the role of MSCs along with the temporal dressings of α-Terpineol (αT)-loaded hydrogel in full-thickness acid burn wounds. We developed a skin substitute comprising of hybrid PVA/ Tapioca starch-based hydrogel loaded with αT (10 µM) and examined its synergistic effect with a single dose of MSCs. A rat model of a full-thickness acid burn wound was developed and characterized. MSCs were isolated and identified through immunophenotyping and trilineage differentiation. *In vitro* skin compatibility of hydrogels was tested on skin fibroblasts, showing 90% cell viability with αT-loaded gels. Further, skin sensitivity analysis on healthy rats revealed no inflammatory response. After confirming their non-cytotoxicity, the hydrogels were topically applied to the wound site for one week (5 h/day), followed by MSC transplantation after 48 h. Macroscopic wound analysis over one month revealed extensive tissue damage and incomplete regeneration in the untreated group, whereas wound dressings with blank and αT-loaded hydrogels improved the healing process. Moreover, a remarkable healing pattern was observed when MSCs and hydrogels were used in combination. As a result, αT-loaded gel + MSCs showed significant ($p \leq 0.05$) wound closure than the blank gel + MSC groups. These results were further validated by histological, gene expression, and immunohistochemical analyses. The study concludes that the hydrating properties of αT-loaded hydrogels provide a cooling effect, maintaining a moist wound environment, while αT accelerates healing by reducing inflammation and oxidative stress. The regenerative effects were further amplified by MSC transplantation, which promoted ECM deposition and re-epithelialization. This combined approach holds great potential for improving burn wound management in clinical applications.

Keywords: *MSCs, α-T-loaded hydrogels, Acid burn wound, Wound dressings, Wound healing.*

OP-60

Chitosan-Sodium Percarbonate-Based Hydrogels with Sustained Oxygen Release Potential Stimulated Angiogenesis and Accelerated Wound Healing

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ABSTRACT

The prolonged hypoxic conditions hinder chronic wounds from healing and lead to severe conditions such as delayed re-epithelialization and enhanced risk of infection. Multifunctional wound dressings are highly required to address the multifaceted challenges of chronic wounds. Herein, we report polyurethane-coated sodium per carbonate-loaded chitosan hydrogel ($CSPUO_2$) as a multifunctional dressing to treat chronic wounds. The hydrogels (Control, CSPU, and $CSPUO_2$) were prepared by freeze gelation method and the developed hydrogels showed high porosity, good absorption capacity, and adequate biodegradability. The release of oxygen from the $CSPUO_2$ hydrogel was confirmed by an indirect method of the increase in pH and a sustained oxygen release was observed over the period of 21 days, due to polyurethane (CSPU) coating. The $CSPUO_2$ hydrogel exhibited around 2-fold increased angiogenic potential in CAM assay when compared with Control and CSPU dressing. $CSPUO_2$ also showed a good level of antibacterial efficacy against E. coli and S. aureus. In a full-thickness rat wound model, $CSPUO_2$ hydrogel considerably accelerated wound healing with exceptional re-epithelialization granulation tissue formation, fewer inflammatory cells, and improved skin architecture, highlighting the tremendous therapeutic potential of this hydrogel when compared with control and CSPU to treat chronic diabetic and burn wounds. Overall, the developed approach provides multifunctional wound dressing to address multiple issues of chronic wounds. In addition, due to the simple process of production and application, the developed hydrogel might have great potential to be applied in clinical settings to treat burn and diabetic wounds.

OP-61

Interaction Between *Gossypium hirsutum* Calmodulin-like Protein 11 (CML11) and Geminivirus-Encoded Proteins using Bioinformatics and Molecular Approaches

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ABSTRACT

Plant viruses are a major menace to crops worldwide, causing devastating diseases that impact food production and ecosystems globally. Despite advances in computational biology, the interactions between plant viruses and their hosts remain poorly understood. We develop a strategic framework using multiple protein binding and interface techniques to elucidate the complex interactions between Geminiviridae viruses and their host cells. Our approach revealed a strong binding affinity between the transcriptional activator protein (TrAP/C2) of CLCuKoV and CLCuMV, and the calmodulin-like protein 11 of Gossypium hirsutum (Gh-CML11). The binding affinity between TrAP and Gh-CML11 was found to be strong, as indicated by a higher negative value for the change in Gibbs free energy. Moreover, gene ontology and nuclear localization signal analyses revealed that TrAP localizes to the nucleus in association with Gh-CML11, facilitating virus infection. interaction prediction and docking analyses provide evidence that both full-length and truncated C2 proteins exhibit strong binding affinity with Gh-CML11. This computational data was further validated with molecular results obtained from yeast two-hybrid, BifC (Bimolecular fluorescence complementation system), and pull-down assay. Furthermore, this study investigates the effects of full-length and truncated TrAP on plant cellular processes. This study presents the first comprehensive analysis of the interaction between cotton CML protein and the transcription activator protein encoded by begomoviruses.

Keywords: *Gossypium hirsutum, Geminivirus, Protein-protein interaction, Bioinformatics, Yeast two hybrid system, Bimolecular fluorescence complementation system.*

OP-62

Discovery of Hydrazone Derivatives as Potent Inhibitors of Beta Secretase 1 for the Management of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease, a neurodegenerative disorder, is a major healthcare concern due to its profound effects on brain function, associated with the build-up of amyloid-β (Aβ) peptide as amyloid plaques

in the brain. This excessive buildup of Aβ peptides is attributed to the uncontrolled activity of a rate limiting enzyme namely beta secretase 1 (BACE1). By inhibiting this enzyme, the formation of Aβ can be limited; therefore, the disease can be prevented. In this regard, novel beta secretase 1 inhibitors (5a-o) have been synthesized and evaluated. Among these inhibitors, 5c, 5d, 5g and 5i presented good inhibitory potential; however, 5d was the most potent of all with an IC_{50} value of 2.81 ± 0.02 µM. Molecular docking evaluation via BioSolveIT suit predicted that the binding energy of 5d in complex with beta secretase 1 was -7.4 kcal/mol and its binding affinity was in micromolar range indicated by HYDE, SeeSAR. Moreover, pre-clinical investigation via SwissADME proved its druggable properties and rendered it specific with no PAINS alerts. At last, it was observed that 5d obstructs the binding site of beta secretase by interacting with residues among which Tyr71 is of prime importance because it is the part of beta secretase 1 flap. This flap has a role in promoting the closed confirmation of beta secretase 1 upon interaction with compound. Consequently, 5d is the most potential inhibitor of beta secretase 1 in Alzheimer's disease but in vivo analysis it is required to further validate the results.

Keywords: *Alzheimer's disease, beta secretase 1, drug discovery, pre-clinical evaluation, synthesis.*

OP-63

Genomic Profiling of Autism Spectrum Disorder in Pakistani Children using Bioinformatics Tools

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ABSTRACT

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that manifests as insufficiencies in social interaction, challenges in communication, and the presence of repetitive behaviors. The pathogenesis of this condition involves various genetic variables, although its etiology remains intricate. Genetic variations may play a role in various physiological processes, such as oxidative stress, immune response, neural development, and synaptic function. The primary objective of this study is to investigate the underlying biochemical and molecular mechanisms responsible for the differential expression of genes associated with Autism Spectrum Disorder (ASD) within Pakistani children. The study will involve the collection of peripheral venous blood samples from a cohort of individuals diagnosed with Autism Spectrum Disorder (ASD) and a control group of individuals without ASD who are matched in terms of age. Advanced molecular techniques will be utilized to

conduct genome-wide expression profiling of these collected samples. After conducting bioinformatics analysis, a selection of Differentially Expressed Genes (DEGs) that exhibit significant variations in expression levels between the two groups will be identified. Additional analysis of the differentially expressed genes (DEGs) will provide a deeper understanding of the enrichment of pathways associated with neuronal signaling and plasticity. This highlights the potential significance of these genes in influencing the fundamental symptoms of Autism Spectrum Disorder (ASD). Functional annotation will be conducted to assess the participation of immune-related processes, thereby indicating a potential association between immune dysregulation and Autism Spectrum Disorder (ASD) within Pakistani children. The data collected will be organized in Microsoft Excel, and the statistical software SPSS V26.0 will be utilized for analysis. The findings of our study will emphasize the importance of population-specific genetic factors, as different ethnic groups may exhibit distinct molecular pathways associated with Autism Spectrum Disorder (ASD). Furthermore, enhancing our comprehension of the pathophysiology of Autism Spectrum Disorder (ASD) can potentially lead to the development of targeted therapeutic approaches tailored to the unique needs of the Pakistani population. The identification of specific genes, pathways and biomarkers associated with Autism Spectrum Disorder (ASD) represents a significant advancement toward achieving a more personalized approach to diagnosing and treating this condition. This discovery holds promise for further exploration of functional investigations and potential applications in clinical practices for Pakistan.

OP-64

Molecular Recognition of Salivary Alpha-Amylase via Fullerene-Porphin Complexation in Dental Caries Diagnosis

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ABSTRACT

Salivary α-amylase is the most abundant protein in human saliva and plays a crucial role in dental caries development by binding to Streptococcus and other bacteria via surface-exposed α-amylase-binding proteins. Its detection in saliva can serve as a valuable bioindicator for assessing caries risk. In this study, we present a facile photochemical sensing strategy based on the tailored properties of 5,10,15,20-Tetrakis(4-hydroxyphenyl)-21H,23H-Porphine (TPPOH) and Fullerene C60 complex. In

principle, starch-coated Fullerene C60, via charge-transfer interactions, quenches the fluorescence emission of TPPOH as confirmed by UV-Vis absorption and fluorescence spectroscopy studies. To ensure structural and functional integrity, starch-coated C60 was thoroughly characterized using Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), optical microscopy, thermogravimetric analysis (TGA), and zeta potential analysis. The developed sensing platform exhibited a linear fluorescence response to α-amylase concentrations ranging from 0.001 to 0.1 Units/mL, with a detection limit (LOD) of 0.001 Units/mL. Further, the applicability of the method was validated in artificial saliva, demonstrating quantitative recoveries in the range of 95–100%. In addition, the practical feasibility of the assay was verified using real saliva samples from individuals across various age groups ranging from 6 to 57 years. Given its sensitivity, specificity, and cost-effectiveness, the proposed method holds significant potential as an alternative analytical tool for early caries detection and risk assessment, potentially reducing the cost of professional preventive measures and treatments.

Keywords: *Dental caries, Saliva, Alpha-amylase, Photochemical, Diagnosis.*

OP-65

Cancer Cell-Type-Dependent Modifications of Metastatic Parameters by SLIT2-ROBO1 and RHOA cAMP Signaling in Response to TGFβ1 and FGF2

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ABSTRACT

The epithelial to mesenchymal transition (EMT) is a multistep process involving structural and functional alterations that are required for cancer metastasis, as well as loss of epithelial markers (e.g., E-cadherin/CDH1) and gain of mesenchymal markers (e.g., N-cadherin/CDH2, vimentin/VIM). Pathological events modify cell-cell interactions, cell-matrix adhesion, and extracellular matrix integrity, leading to cell migration, evasion from the primary tumor and augmented invasiveness in the metastatic niche. This transformation is modulated by multiple paracrine factors (e.g., chemokines, growth factor), as well as SLIT2-ROBO1 signaling that collectively regulate the expression of RHO GTPases (e.g., RHOA) and EMT marker genes. Yet, the roles of SLIT proteins in cancer remain enigmatic. In some cancer types, SLIT2 is anti-tumorigenic, while in other cancers it contributes towards the metastatic phenotype. Here we investigated the ambivalent metastatic activity of SLIT2 by analyzing how cAMP/RHOA signal transduction modulates SLIT-ROBO controlled metastatic parameters in response to the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine) and paracrine factors (TGF-β/TGFβ1 and FGF2). Upon SLIT2 administration cell migration and

proliferation increases in colon cancer cells and decreases in cervical cancer cells, while altering cell morphology and proliferation in both cancer types. These effects are reinforced by TGF-β/TGFβ1 and FGF2, but attenuated by elevation of cAMP with IBMX, depending on the cancer cell type. Our data indicate that SLIT2 represents a potential biomarker for cancer diagnosis, prognosis, and therapy.

Keywords**:** *SLIT2, ROBO1, RHOA, cAMP signaling, apoptosis, colorectal cancer cells, cervical cancer cells, TGFβ1, FGF2, IBMX.*

OP-66

Anticancer Effects of Chrysin in Promoting Apoptosis in T47D Tumor Cells

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ABSTRACT

Cancer is a major public health disease present in the whole world. New cases are account 14.1 million and the mortality proportion is 8.2 million in 2012. Estimate that 22.2 million new cases will be projected due to this continuous enhancement. We are estimating that 13.2 million death rate per year 2030 in whole world. Chrysin is a natural flavonide which present in honey, propolis, plant extract, fruits, vegetables, nuts, tea and wine. Chrysin is a flavones present in some plants *Passiflora caerulea, Passiflora incarnate Oroxylum indicum, Matricaria chamomilla* and feature items *Pleurotus ostreatus* and nectar. Chrysin is used as antioxidant and has anti-ageing, anticancer and anti-inflammatory properties. Chrysin inhibit the growth of uncontrolled cells in the human body. Chrysin introduce the cell death in body. In present studies we estimated that the chrysin is accumulated in the breast cancer T47D and MCF7 cell lines. It increases cytotoxicity in cancerous cells without being harmful to the normal cells. The use of chrysin loaded with nanoparticles such as PLGA-PEG increase its solubility and bioavailability. When T47D cell lines are treated with chrysin loaded with PLGA-PEG, increases the inhibition of cell proliferation in a dose-dependent manner and improves the cell growth inhibition. Chrysin or silibinin are two herbal products that have anti proliferative effects on T47D cancerous cells of the breast and alone they inhibit the proliferation in time and dose dependent manner.

Keywords: *Apoptosis, Chrysin, flavonoids, Breast cancer, Colon cancer, In vivo, Sall4, T47D.*

OP-67

Ligand-Based Drug Design Studies of Different Flavonoids as a Potential Inhibitor of BCR-ABL

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ABSTRACT

BCR-ABL is a gene formed by the breakdown and fusion of pieces of two different chromosomes. It causes the production of abnormal blood cells to produce much protein called tyrosine kinase that promotes cancer by allowing uncontrollable cell growth. BCR-ABL fusion genes are found in most patients with chronic myelogenous leukemia (CML), which is an uncommon type of bone marrow cancer. To inhibit Tyrosine kinase causing BCR-ABL activity, there were several clinical inhibitors discovered. Imatinib was discovered as a first-generation drug that inhibits the Tyrosine kinase activity. Dasatinib, nilotinib, bosutinib, ponatinib, and befetinib are identified as second and third-generation inhibitors. In our study, we have identified some polyphenols such as Baicalein, Erysubin A and others have greater potency to act as capable inhibitors than clinical compounds. Dasatinib and Bosutinib are used as control in our current study. To this context, we study 13 flavonoids as a ligand using structure based virtual screening from PubChem database to identify as inhibitors against BCR-ABL oncogene. Further studied for extra precision, molecular docking was performed using AutoDock Vina which shows binding affinity of compounds between -6.9 to -10.5 kcal/mol. Erysubin A and Bacialein were most suitable flavonoids with binding affinities of -10.5 and -10.0 kcal/mol respectively, compared to clinical inhibitors, i.e., Bosutinib and Dasatinib, having affinities of -7.9 and -8.7 kcal/mol, respectively. Further studies have shown that Erysubin A and Baicalein have significant interactions with various residues of most sustainable amino acids. Hence, these results supported the idea that these polyphenols are potential compounds with an ability to inhibit cancer by disrupting the fusion gene activity.

Keywords: *Molecular docking, Flavonoids, Bcr-Abl Inhibitor, Drug Discovery, Cancer Treatment*

OP-68

Alpha-2-macroglobulin (A2M) and Alpha-2-HS glycoprotein (FetuA), Potential Markers of Renal Cell carcinoma (RCC), an Insight From Proteome Profile of Cancer Tissues"

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ABSTRACT

Renal cell carcinoma (RCC) is characterized as the most common neoplasm of the human kidney among the top fifteen most diagnosed tumors, encompassing multiple sub histologies with definite

genomic, clinicopathological, genomic and proteomic features. Proteomic technologies enable the detection and quantitation of protein profiles associated with RCC to delineate the dysregulated expression of various proteins involved in multiple cellular processes. In this study, which is novel for the local population, we employed liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis to characterize proteome profiles of the tissue, and the serum samples obtained from renal cell carcinoma patients. Five paired (RCC and adjacent normal) and 2 pooled sets of samples were utilized. Out of the 3,167 identified proteins from MS spectra 78 of interest (p value ≤ 0.05; 0.9 % protein decoy FDR; 0.07 % peptide decoy FDR; fold change ≥ 1) were selected through scaffold analysis with up regulated expression of 42 proteins in tumor along with 36 downregulated proteins. From the panel of 13 proteins i.e. ACTG2, A2M, FETUA, CAP1, OSTF1, RL32, PDLI1, GBB1, MAP1B, RL30, PIMT, C163A, SC22B and SMD3 that were not previously reported in RCC, two proteins; Alpha -2-macroglobulin (A2M) and Alpha-2-HS glycoprotein (FetuA) were subjected to validation through multiplexed analysis by Luminex and immunohistochemistry. We found upregulated expressions of A2M (2.6 folds) and FetuA (1.6 folds) along with decreased serum excretion (P=0.0061) (P=0.0002) in RCC patients, respectively. The expression was validated through histochemical studies of the tissue samples. Further studies on larger sample size and rich validity cohort shall elucidate the role of A2M and FetuA to serve as novel therapeutic interventions for RCC.

Keywords: *RCC, Pakistan, A2M, LCMS/MS.*

OP-69

Nanoparticles-Based Delivery System For CRISPR-Mediated Gene Disruption And To Deliver Crispr Cas9 Components To Cancer Cells

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ABSTRACT

Cancers are getting harder to treat because of poor editing efficiency within the tumors and the damage caused by conventional delivery methods. Our study details a system that

comprises lipid nanoparticles (LNP) with a newly designed amino ionizable lipid for enhanced delivery of CRISPR-Cas9, in so doing breathing life to the notion of gene editing. The direct use of CRISPR-LNPs targeting PLK1 (injecting sgPLK1-cLNPs) into the brain of an advanced glioblastoma led to more than 65% gene editing within the body, this also led to a 45% decrease in the rate of tumor proliferation along with a 25% increase in overall survival. For metastatic tumors, theLNPs were additionally modified by several means and made for antibody-mediated targeting. EGFR targeting led to significant accumulation of the clipPLK1 containing LNPs to disseminated ovarian tumor implants, resulting in approximately 75%. This greatly inhibited the rate of tumor growth of 1408 and increased longevity by approximately 70%. This study paves the way for utilizing nanoparticles containing CRISPR to easily and effortlessly target and edit cancerous cells. This work will profoundly alter the landscape of cancer therapy and gene editing techniques.

Keywords: *Nanoparticles, Cancer cells, CRISPR, EGFR, CAS9.*

OP-70

HDLBP Contributes to Goose Fatty Liver Development by Modulating Oxidative Stress and Inflammatory Responses

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ABSTRACT

Goose fatty liver is a physiological fatty liver induced by carbohydrate-rich feeding, distinguished by its resistance to inflammation despite lipid accumulation. Understanding the function of HDLBP in regulating oxidative stress and mitochondrial function may reveal key mechanisms underlying its unique protective adaptation. The aim of this study is to validate the organelle-specific distribution of high-density lipoprotein binding proteins (HDLBP) within cell via in-vivo and animal cell line models and its correlation with hepatic oxidative stress and inflammatory responses during the formation of goose fatty liver. For in vitro experiments primary hepatocytes isolated from 23-day-old goose embryos were transfected with an HDLBP overexpression vector. Experimental model of Fourteen 70-day-old Landes male geese divided randomly into a control group (ad libitum feed) and an overfed group (force-fed for 20 days weighing 3.72kg and 3.71kg respectively. Immunofluorescence and immunoblotting assay were used to confirm the mitochondrial localization of HDLBP (mHDLBP) and in vivo detection. Overfeeding significantly reduced the protein abundance of total cellular HDLBP (wHDLBP) and mitochondrial HDLBP (mHDLBP) ($P < 0.01$). In primary hepatocytes, HDLBP overexpression led to increased mHDLBP protein levels ($P < 0.05$), elevated oxidative stress markers, including reactive oxygen species (ROS) and malondialdehyde (MDA) ($P < 0.05$), and decreased mitochondrial membrane potential and antioxidant enzyme activities (T-SOD, GSH-PX; $P < 0.05$).

Transcriptomic analysis revealed HDLBP overexpression upregulated genes involved in immune and inflammatory pathways, such as IL1R1, TNFSF10, LTC4S, NCF1, and KDR, while overfeeding suppressed their expression in vivo ($P < 0.05$, 0.01, or 0.001). These findings validate HDLBP influences mitochondrial function, oxidative stress, and inflammatory responses, potentially acting as a regulatory factor in goose fatty liver development. The observed downregulation of HDLBP in fatty liver may serve as a protective mechanism against inflammation. This unique feature of geese fatty liver distinguishes physiological hepatic steatosis from pathological non-alcoholic fatty liver disease in mammals.

Keywords: *HDLBP (High-Density Lipoprotein Binding Protein), Goose Fatty Liver, Oxidative Stress, Mitochondrial Function, Inflammatory Response.*

OP-71

Epigenetic Inactivation of MST1 in Tongue Cancer: DNA Methylation as a Diagnostic and Therapeutic Target

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ABSTRACT

To investigate the DNA methylation status of the mammalian sterile 20-like kinase 1 (MST1) promoter region, its mRNA expression levels, and their associations with tongue squamous cell carcinoma (TSCC). The study included blood samples from 42 TSCC patients and 100 healthy controls. DNA extraction, bisulfite conversion, methylation-specific PCR, and electrophoresis were performed to analyze the methylation status of the MST1 promoter region. RNA extraction and real-time reverse transcriptase PCR were conducted to evaluate MST1 mRNA expression in CAL-27 (TSCC cell line) and HGF-1 (normal oral cell line). Statistical analysis included chi-square tests and odds ratio (OR) estimation to determine the association between MST1 promoter methylation and TSCC. Methylation of the MST1 promoter region was detected in 93% of TSCC samples and in both cell lines. A statistically significant association was observed between hypermethylation of the MST1 promoter and TSCC (chi-square = 13.7, P-value = 0.00001). Subjects with hypermethylated MST1 promoters had an approximately 8-fold increased risk of developing TSCC (OR = 7.98, CI = 2.30–27.58). Gene

expression analysis revealed that CAL-27 cells had a 0.39-fold lower MST1 expression compared to HGF-1 cells, suggesting significant down-regulation in TSCC. Hypermethylation of the MST1 promoter region is strongly associated with TSCC and contributes to the down-regulation of MST1 expression. These findings suggest that DNA methylation of MST1 can serve as a potential biomarker for TSCC diagnosis and as a therapeutic target. Incorporating epigenetic insights into clinical practice may enhance the understanding of TSCC progression and improve treatment strategies.

Keywords: *Epigenetics, DNA Methylation, Promoter Region, MST1, Tongue Squamous Cell Carcinoma.*

OP-72

Human Blood and Saliva DNA Degradation Associated with Artificial Ultraviolet And Solar Radiation As A Function of Exposure Time

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ABSTRACT

Forensic investigations utilizing DNA analysis have advanced rapidly. Blood and saliva are the most found biological samples at crime scenes. However, these samples are often exposed to various environmental conditions, such as natural solar radiation, artificial ultraviolet (UV) radiation, temperature fluctuations, humidity, and pH changes, all of which can lead to DNA degradation. This study aimed to investigate the degradation of human blood and saliva DNA when exposed to artificial ultraviolet and natural solar radiation over time. For the experiment, 10 µl of human blood and saliva were subjected to artificial UV light at 3.2 cm in a laminar flow hood (wavelength: 235.7 nm) and natural solar radiation (UV index = 9, temperature: 38°C in Mardan and Peshawar, and 29°C in Swat) across three districts of Khyber Pakhtunkhwa (Swat, Mardan, and Peshawar). The exposure time was set at intervals of 20 minutes, up to a total of 120 minutes. DNA was then extracted using the manual PCI method and amplified using various primers, including HVR, mitochondrial control region, gender identification primers (AmelT, HumAmel), and pigmentation-related primers (HERC2, OCA2, IRF, TYR, SLC24A2, and SLC45A2). The results indicated that artificial UV radiation caused significant DNA degradation in blood samples after 40 minutes of exposure. In contrast, under natural solar radiation, DNA bands were bright in samples exposed for 20 and 40 minutes but gradually faded in samples exposed for longer durations. For saliva samples, no DNA bands were observed in those exposed to artificial UV radiation. However, saliva samples exposed to natural solar radiation in Swat showed bright DNA bands even after 120 minutes of exposure. The best amplification results were observed with the SLC45A2 and TYR primers. The findings suggest that DNA degradation is more pronounced in samples exposed to artificial UV radiation compared to natural solar radiation.

Furthermore, in the comparison of blood and saliva, saliva was found to be more susceptible to degradation than blood.

OP-73

Beyond the Trauma: Epigenetics, PTSD, and the Emergence of a Reciprocal Causality Paradigm in Psychiatric Genetics

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ABSTRACT

Post-traumatic stress disorder is a debilitating mental health concern that affects approximately 3.9 % of the global population. The traditional linear approach to PTSD overlooks the dynamic relationship between PTSD and epigenetics. Epigenetics, as a biomarker of PTSD, elucidates the modifications in the genes and neurobiological abnormalities specific to individuals, groups, and successive generations. The epigenetic modifications caused by trauma exposure can impact individuals with less prior vulnerability. By adopting this reciprocal paradigm shift, the etiology of PTSD based on heritability, epigenetic modifications, and genetic predisposition can be investigated to curate an integrated approach to prevention and treatment. By the incorporation of personal experience, narrative review of literature, and conceptual reasoning, this perspective article will investigate the reciprocal causality relationship between PTSD and epigenetics. A comprehensive narrative literature review revealed a consistent pattern of environment-sensitive epigenetic modifications coinciding with the development of PTSD. Consistent evidence of a biological imprint of trauma and greater propensity to encounter trauma in offspring of trauma-exposed parents was revealed. Sex-specific loci in females were a risk factor of PTSD. Trauma exposure induced neurobiological abnormalities, causing gene regulatory modifications. These findings emphasize the importance of a framework of preventative and treatment interventions informed by the reciprocal causality paradigm with epigenetics as a biomarker. A detailed analysis of available literature presents a feedback loop encompassing the intricate interplay between genetic predisposition, epigenetic alterations, and environment. Causality relationship endorsement with emphasis on personalized targeted interventions and epigenetic therapies is required to enhance patient outcomes. Ancestral trauma assessment in clinical evaluation for PTSD and research in population-specific longitudinal studies can pave the way for proactive interventions. The reciprocal causality narrative, that elucidates the induction of epigenetic alterations

because of trauma exposure, influences susceptibility to PTSD and can enhance resilience when incorporated through actionable measures.

Keywords: *PTSD, Epigenetics, Trauma, Therapeutic targets, Neurobiological abnormalities.*

OP-74

From Possessiveness to Peace: The Psychology of Othello Syndrome and the Healing Power of Soul Satisfaction

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ABSTRACT

Othello Syndrome is one major psychological phenomenon that is destroying relationships (among partners) and the mental well-being of people suffering from it all over the world and is often overlooked by the researchers' community. Focusing on the idea that individuals affected by this condition may not require medication but instead need emotional peace and soul satisfaction for healing. In this case, the individuals who experience this syndrome intermittently feel fine for some periods and then relapse back into its symptoms. This research offers insights into alternative, non-pharmaceutical approaches for individuals affected by Othello Syndrome. Ultimately, it offers a pathway to psychological recovery and emotional balance for those affected by this syndrome. By doing a narrative review of available literature driven by conceptual analysis and integrated with personal experience, this study aims to understand the psychological and emotional challenges faced by individuals struggling with this syndrome and how to deal with it the right way. It was confirmed that individuals with Othello syndrome often experience significant psychological distress characterized by heightened anxiety, intrusive thoughts, and pervasive distrust in relationships. Soul satisfaction emerged as a key pathway to long-term recovery and relationship restoration, which was properly carried out by spiritual therapies and psychotherapies in most of the patients. In a nutshell, the concept of soul-satisfaction achieved through spiritual growth, self-reflection, and fulfilling relationships-emerges as a powerful pathway to healing this syndrome. By fostering inner peace and purpose, individuals can mitigate the psychological effects of Othello syndrome, leading to more harmonious relationships and improved mental health. Everyone should work together to counter this syndrome the right way and bring peace to patients all over the world.

Keywords: *Othello Syndrome, Soul-satisfaction, Spiritual therapy, Psychotherapy.*

OP-75

Our Skin Biology Predicts Whether We are Going to Hell or The Heaven: A Scientific Link Between Our Spiritual and Physical Existence

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ABSTRACT

A person's true identity is in his face. So, we recognize people by observing the color and shape of their faces. It is a fact that our face is in a continuous process of alteration in colour and shape throughout our life. The most widely accepted reasons for these alterations are considered to be our environment. The present study was aimed at finding important reasons for these alterations other than our environment. Volunteers of different ages, genders, social backgrounds and religious tendencies were chosen for the study. The main parameters observed and analysed in the study were changes in skin colour and the design of the human face. The study revealed that the human face changes both in colour and shape in a matter of minutes or even seconds within the same day under the same environmental conditions. More interestingly, these changes were observed to be directly related to human ethical and religious activities.

Keywords: *Skin colour, Face beauty, Prediction of the future.*

OP-76

Nutrigenetics: Exploring the Genetic Influence on Vitamin D Levels in Pregnant Women of Pakistan

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ABSTRACT

Vitamin D deficiency is a widespread health concern affecting individuals across all age groups. Pakistan, though rich in sunlight, has long struggled with high rates of vitamin D deficiency, yet many remain unaware of its epidemiology and underlying causes. Our study explored this issue from both nutritional and genetic perspectives through a cross-sectional study conducted in rural Jehlum and urban Karachi. We aimed to evaluate the prevalence of vitamin D deficiency in pregnant women and neonates in Pakistan and assess the possible association between serum 25-hydroxyvitamin D [25(OH)D] concentrations and vitamin D binding protein (Gc) genotypes. A total of 390 women and

266 neonates were recruited from urban and rural sites. Serum 25(OH)D levels were measured using DiaSorin immunoassay, while Gc genotypes were identified using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Statistical analysis was performed using Chi-square, One-way ANOVA and Linear regression. Our findings revealed alarmingly high deficiency. In urban Karachi, 99.5% of women and 97.3% of neonates were vitamin D deficient (serum 25(OH)D < 50 nmol/L). In rural Jehlum, 89% of women and 82% of neonates were deficient. No significant association was found between Gc genotypes and serum 25(OH)D concentrations in either group. Vitamin D deficiency is highly prevalent among Pakistani women and their neonates. However, Gc genotypes do not appear to influence serum 25(OH)D levels. These findings underscore the need for urgent public health interventions to address vitamin D deficiency in Pakistan.

Keywords: *25-Hydroxyvitamin D, Gc genotypes, Gc1S, Gc2, neonates, Pregnant women.*

OP-77

Antiadipogenic Potential of Cissus Quadrangularis

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ABSTRACT

Herbal alternatives for weight loss present a promising solution, offering effective reductions in body mass index (BMI) without the severe side effects associated with conventional drugs. Among these, Cissus quadrangularis (CQ) has emerged as a potent candidate for weight management. Our laboratory research highlights the anti-adipogenic properties of CQ solvent fractions and their potential role in combating obesity. Through comprehensive analysis of cell viability, growth, proliferation, and metabolic activity in the 3T3-L1 cell line, we identified non-cytotoxic, metabolically active concentrations of Ethyl Acetate (CQ-EA), Butanol (CQ-B), Dichloromethane (CQ-D), and Hexane (CQ-H) fractions. These fractions were administered during the differentiation of 3T3-L1 cells into adipocytes (adipogenesis). Subsequent gene expression profiling revealed that CQ fractions significantly downregulated key adipogenic and lipogenic markers, including peroxisome proliferator-activated receptor-gamma (PPAR-γ), adiponectin (ADIN), fatty acid binding protein-4 (FAB4/aP2), and leptin (LPN). The anti-adipogenic potential of CQ fractions was further corroborated by reduced triglyceride levels and decreased neutral lipid accumulation, as evidenced by Oil Red O (ORO) staining. A reduction of 40–50% in triglycerides and neutral lipids was established as the benchmark for an effective anti-obesity agent. Cissus quadrangularis demonstrated remarkable efficacy in inhibiting adipocyte differentiation and lipid synthesis, positioning it as a potent anti-obesity agent capable of modulating cytokine production, including adiponectin and leptin. Western blot analysis

confirmed lower protein expression levels of PPAR-γ, FAB-4, and fatty acid synthase (FAS), further validating these findings. Finally, Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the CQ-H fraction, which exhibited the most pronounced anti-adipogenic effects, provided additional insights into its bioactive components.

Keywords: *Stem Cells, Cell Culture, Natural Herb, Molecular Biology, Anti-Obesity, Solvent Fractionation.*

OP-78

Biofabricated AgZnO Nanoparticles from Citrus limon Peel Extract: Assessment of Antioxidant, Antimicrobial, and Wound Healing Activities

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ABSTRACT

Lemon, scientifically known as Citrus limon (Rutaceae), is an important medicinal plant utilized mostly for its alkaloids. Citrus flavonoids exhibit strong antibacterial, antidiabetic, antifungal, anticancer and antiviral activities. Flavonoids can alter enzyme activities, restrict cell proliferation, and act as direct antioxidants and free radical scavengers. The present study's objective was to evaluate the antioxidants, antibacterial, and in vivo wound healing properties of AgZnO NPs made using a practical green chemistry technique with Citrus limon extract as a capping agent. The physicochemical characteristics of AgZnO NPs were investigated using UV-vis, FTIR, SEM, EDX, and XRD to ascertain their structural properties. The antioxidant activity was calculated by DPPH free radical scavenging assay and FRAP assay. Ascorbic acid was served as standard for both assays. Results showed that the AgZnO NPs have higher antioxidant activity than standard ascorbic acid. To evaluate the antibacterial activity of AgZnO NPs, Ciprofloxacin and Cifixime were employed as positive controls against five different bacterial strains. Among these strains, the highest inhibition zone 13±1.8 mm was observed against Staphylococcus aureus with concentration of 100 µl AgZnO NPs. In vitro cytotoxicity was done to calculate the percentage of viable cells by using the HepG2 cell lines. Results showed that AgZnO NPs displayed 20% viability against cancerous cells. For the evaluation of in vivo wound healing activity, different formulations of nanoparticles loaded with carbopol gel were prepared and the evaluation of these formulations was done by physical examination (color, homogeneity, and pH). Additionally, rats were categorized into four groups for in vivo study and six rats were assigned to each group. Results showed that wound healing in rats treated with 1.5% AgZnO NPs gel formulation was significant rather than control. Histopathological investigations were conducted to

assess fibrosis progression on the skin of rats on the 10th day, focusing on the observation of fibroblast and macrophage distribution to determine the extent of spread. Bimetallic AgZnO nanoparticles were anticipated to possess antioxidant, antibacterial and wound healing properties. The findings of this study have the potential to contribute to the development of novel therapeutic interventions and provide insights into the application of green chemistry approaches in the production of nanoparticles.

Keywords: *Citrus limon, AgZnO nanoparticles, green synthesis, antioxidant activity, DPPH assay, FRAP assay, antibacterial activity.*

OP-79

Anti-Convulsant Activity of 3H-Quinazoline-4-One Derivatives: Design, Synthesis, and Biological Evaluation

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ABSTRACT

Epilepsy is a chronic neurological disorder in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally that may remain localized (focal epilepsy) or become widespread (generalized epilepsy). The term epilepsy is derived from the Greek word epilepsia, which means "falling sickness" and can be called "seizure", "ictus", or "convulsion". Quinazoline derivatives have been involved in the design of new anti-convulsant and CNS depressant agents. The anti-convulsant and CNS depressant activity is due to the presence of aromatic/aliphatic group at position 2 and substituted aromatic ring at position 3. The synthesized compounds were also tested for the antioxidant activity, compound Zc, Zd and Ze shows promising results. These compounds also exhibit good bonding affinity with carbonic anhydrase II and revealed a manifestation of structure activity relationship (SAR). The compound Zc, Zd and Ze exhibited significant antioxidant activity having IC_{50} value of 7.48, 4.85 and 10.28 µg/ml respectively. Anti-convulsant activity was performed in-vivo by utilizing mice, compounds which show prominent antioxidant activity showed good anti-convulsant activity. These compounds showed their anti-convulsant activity by removing tonic-clonic seizures induced by PTZ. The in-vitro enzyme inhibition and computational studies have indicated that these compounds can become good drug candidates if they may be screened against more protein/ enzyme targets. Thus, 3H-quinazoline-4-one is an important nucleus and can be utilized to develop innovative drug molecules that may have good pharmacological activity.

Keywords: *Epilepsy, CNS depressant, Quinazoline, Tonic-clonic seizures, Computational studies.*

OP-80

In-Silico Drug Discovery of 2-Aminobenzothiazzole for Anti-Ulcer Activity: A Computational and Pharmacological Approach

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ABSTRACT

Benzothiazole has gained worldwide interest among researchers because of its enhanced pharmacological, biological and chemical activities. It is considered as an important pharmacophore and has privileged structure in medicinal chemistry, exhibiting many useful therapeutic activities. Therefore, the present study was designed to synthesize different derivatives of 2-aminobenzothiazole and evaluate their pharmacological potential by virtue of presence of benzothiazole framework. These derivatives were synthesized by three step procedures and characterized with the help of FTIR and 1HNMR. These compounds were then subjected to in vitro and in vivo studies including anti-oxidant, anti-inflammatory and anti-ulcer activity. Among all the synthesized derivatives the highest antioxidant activity was shown by compound 3e with % inhibition of 92.1% and IC50 value of 5.64 µg/ml which was comparable to the positive control ascorbic acid. Two other compounds 3d and 3b also exhibited comparatively good antioxidant activity. The test compound 3e (2 mg/kg) selected based on good anti-oxidant activity, exhibited an excellent anti-inflammatory effect with a significant decrease in paw volume. Among the selected compounds, 3e showed significantly high anti-ulcer activity at 10 mg/kg dose by exhibiting 80% inhibitory effect with ulcer index of 2 ± 0.19. Compound 3b and 3d also showed relatively good anti-ulcer activity at a dose of 10 mg/kg with ulcer index of 3 ± 0.2 and 2.5 ± 0.13 respectively. Further, to check the affinity of synthesized compounds, they were subjected to molecular docking studies and the interactions involved in their binding were also analyzed. Therefore, our results support the further clinical promise of 2-aminobenzothiazole derivatives as a component of therapeutic strategies for the management of gastric ulcer disease. They can be used as lead compounds for further drug discovery.

Keywords: *In-silico, Computational, Anti-ulcer, Molecular docking, Benzothiazole.*

OP-81

New Derivatives of Ibuprofen as Potential LOX Inhibitors; Synthesis, Characterization, In Vitro And In-Silico Studies

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ABSTRACT

N-Acylhydrazones represent a diverse class of organic compounds with notable chemical and biological significance. Their structural framework, featuring both electrophilic and nucleophilic centers, facilitates various molecular interactions. Besides, ibuprofen, a well-known NSAID, contains a chiral 2-arylpropionic acid core, with its pharmacological activity attributed to the (+S) enantiomer. While numerous ibuprofen derivatives have been studied, N-acylhydrazone analogs based on ibuprofen remain largely underexplored. This study focuses on the synthesis and LOX inhibitory assessment of novel ibuprofen-derived N-acylhydrazones. LOX enzymes play a central role in inflammation and disease pathogenesis and serve as critical targets for therapeutic intervention. The synthetic strategy involved esterification, hydrazination, and subsequent condensation with heterocyclic aldehydes and methyl ketones. Biological evaluation revealed potent LOX inhibition, with compound 5e (3-nitrophenyl) demonstrating the highest activity (IC_{50} = 3.6 ± 0.71 µM), followed by 7h (9H-fluorenyl, IC_{50} = 4.0 ± 0.30 µM). Structure-activity relationship (SAR) analysis suggested that electron-withdrawing substituents and extended aromatic systems enhanced inhibitory potential, whereas bulky groups diminished activity. Molecular docking confirmed strong LOX binding, with 5e and 7h exhibiting high binding affinities (-10.7 and -10.8 kcal/mol, respectively). Furthermore, molecular dynamics simulations indicated that these lead compounds formed stable enzyme-inhibitor complexes. In a nutshell, 5e and 7h emerged as promising candidates for further optimization in anti-inflammatory drug development, potentially offering enhanced efficacy and reduced side effects.

Keywords: *Biological evaluation, lipoxygenase, molecular docking, synthesis.*

PP-01

Integrated Genome-based Breeding for Development of Climate Resilient Crops in Rapid Time

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ABSTRACT

According to food security analysts, global agricultural production must double by 2050 to meet the demands of the increasing world human population. Still, this challenge is further exacerbated by unpredictable climate change. The availability of new climate-resilient crop varieties in rapid time is an uphill task to feeding the global population. Lack of application of new breeding technologies (NBTs) is the major bottleneck for the speedy development of the climate resilient varieties. For rapid increase in yield, the utilization of NBTs including implementation of UAV-based high throughput phenomics, genome–based breeding by design, genomic selection, and genome editing galvanized by speed breeding are the desirable strategies to meet the rising food demand. At NIGAB under the Sino-Pak project all these techniques are being used to improve the crops in a rapid time. Pre-breeding in the form of the discovery of new genes and association of markers with traits through Genome Wide Association Studies (GWAS) has added to the identification of 28 candidate genes for traits-specific breeding. Advancements in NGS have revolutionized genome-based breeding by determining the genomic constitution of parents and offspring for a particular target environment (TE). In this regard, new genomic platforms, PCR-based KASP markers, and HTP have facilitated the use of genomic selection based on genetic variants existing in indigenous cultivars of wheat, rice, and sugarcane. A decade of CRISPR/Cas technology has brought improved nutritional value, disease resistance, and improvement and expansion in crops. Several genes controlling different traits, root architecture, canopy, heat tolerance, and anti-starch and sucrose contents are being edited using CRISPR/Cas. The implementation of integrated genome-based speed breeding technologies has a great potential for accelerating the pace of development of new high-yielding climate-smart varieties in rapid time.

Keywords: *Crop improvement, Speed Breeding, Functional Genomics, Biotechnology, Phenomics, Crops, Gene editing, Climate, GWAS.*

PP-02

Mitigation of Drought Stress by Foliar Spray of ZnO Nanoparticles in Wheat (*Triticum Aestivum L.)*

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ABSTRACT

Drought stress is one of the major abiotic factors that affect plant growth and development. This study investigated the drastic effects of drought on the growth, biochemical and physiological characteristics of wheat and the role of foliar application of ZnO nanoparticles treatment in growth improvement and drought stress mitigation in wheat crops. Wheat cultivar Galaxy-13 was cultivated in pots filled with a uniform mixture of soil, sand, and garden compost, exposed to 50% of field capacity under drought stress and 100% of field capacity under control conditions. 100ppm ZnO nanoparticles were applied (foliar spray) in control as well as drought stress condition. The ZnO nanoparticles, known for their distinctive physicochemical characteristics, can influence plant metabolism and enhance physio-biochemical and yield attributes. The data revealed a significant increase in the fresh and dry weight of shoots and roots with the application of ZnO nanoparticles. These NPs also activated the antioxidant defense system and protected the crops from oxidative damage. In short, ZnO nanoparticles improved the development, physiology, and antioxidant defense of plants, thereby mitigating the detrimental effects of drought. It can be concluded that the application of ZnO nanoparticles may have the potential for growth improvement and reducing the effect of drought stress on wheat (*Triticum aestivum* L.) variety Galaxy-13.

Keywords: *Wheat, Nanoparticles, Drought, Physiology, and Yield.*

PP-03

Synthesis of Nano-Fertilizer by Utilization of Banana Peel Extract and its Impacts on the Growth of Different Plants

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ABSTRACT

Banana peel contains important nutrients that can be recycled into useful products used for different purposes. Banana peel is used to form biostimulant nano fertilizer for agricultural purposes. Extraction of nano fertilizer from the banana peel is the main step of this study. The alkaline solution helps the banana peel for the formation of nano fertilizer. Nano-fertilizer extracts subjected to physical and chemical analyses for characterization. The particle size range of fertilizer is between 18nm-54nm, and the study shows that 39.9 nm is a major nanoparticle, and it contains an average percentage of 36%. Nanofertilizer increases the growth rate in plants. Fourier transform infrared spectroscopy

(FTIR) inspection is used for the recognition of polymeric, inorganic, and organic materials. It used infrared light for sample scanning. Changes and errors in the characteristics sequence of the absorption band show an alteration in the composition of the nanofertilizer. The synthesized nano fertilizer from banana peel contains tryptophan, urea, proteins, citric acid, chelated potassium, amino acids, and chelated iron. Synthesized nano fertilizers applied to pea, chili, and tomato plants. This study aims to observe the germination process of plants increasing or decreases by increasing doses of banana peel extract for crops and to evaluate the effects of nanofertilizer on pea, chili, and tomato plants. The obtained nano biostimulant shows great germination for different crops.

Keywords: *Nanofertilizer, Banana Peel, FTIR, Potassium Hydroxide, Growth Evaluation, etc.*

PP-04

Enhancing Fe/Zn Accumulation in Wheat through Optimized Fe/Zn Fertilization Strategy

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ABSTRACT

Fe/Zn are vital micronutrients for maintaining good human health and their deficiencies can lead to various health concerns. The general population of Pakistan suffers with Fe/Zn deficiencies due to insufficient micronutrient supply. Wheat, the staple crop, contributes significantly to national food security, but faces rising production costs, mainly due to increased expenses for fertilizer, and manure. This study focuses on developing wheat varieties with higher grain yield and enhanced Fe/Zn accumulation. Therefore, field trials with Randomized Complete Block Design (RCBD) experiment were conducted at NIBGE, involving four High Yield Potential Trial (11th HYPT & 10th HPYT)-elite wheat lines from CIMMYT and two locally adapted wheat cultivars; AKBAR-2019 and UROOJ-2022 used as biological control treated with 50% of recommended Fe/Zn fertilizer application. Results indicated that Fe/Zn application reduced plant height but improved/depicted positive responsive behavior for the spike length, leaf area, and grain weight. Total grain yield, on the other hand, was found to be statistically significantly correlated with zinc ($p<0.05$) levels in flag leaves during the grain loading stage irrespective of population types. Vapor pressure deficit (VPD)/leaf to air vapor pressure, an important factor for nutrient uptake and plant health, was highest with foliar Fe/Zn applications, followed by combined and soil-alone applications. Stomatal conductance also depicted a similar trend to VPD. Metal profiling showed an increase in Zn corelated with Mn levels, while Fe, exhibited an inverse correlation with Zn in Fe/Zn applications through soil mode alone, soil and foliar combined, but less differences were observed through foliar mode of Fe/Zn applications. The increased

photosynthetic and transpiration rate observed under foliar Fe/Zn application were likely influenced by the regulation of key metabolites such as oxime methoxyl phenyl, Butanedioic acid, 2-propenoic acid, 2-methyl-, propyl ester, Phenol, 2,4-bis(1,1-dimethylethyl)-, 2-Propyl-1-pentanol, and other. This research highlights the potential for nutrient-enriched wheat varieties through optimized Fe/Zn applications.

Keywords: *Wheat biofortification, Food security, Micronutrient deficiency, Fe/Zn deficiency, Iron Zinc fertilization, metabolite profiling, Elemental analysis, Vapor pressure deficit (VPD), Photosynthesis rate.*

PP-05

Floral Infusion Biotechnological Approach to Sustainable Fragrance

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ABSTRACT

The global fragrance industry relies heavily on synthetic chemicals and unsustainable harvesting practices, leading to environmental concerns and potential health risks. Floral Infusion is an innovative biotechnology solution that leverages plant tissue culture techniques to produce natural fragrances without harming the environment. By cultivating plant cells in controlled conditions, we can extract high-quality essential oils and aromatic compounds efficiently and sustainably. The global fragrance market is experiencing significant growth, driven by increasing consumer demand for natural and sustainable products. While the market is dominated by synthetic fragrance manufacturers, there is a growing niche for natural and organic fragrances. Floral Infusion's sustainable production methods position it as a strong competitor. Plant tissue culture involves cultivating plant cells in a sterile environment with a nutrient-rich medium. By manipulating the growth conditions, we can induce the cells to produce specific secondary metabolites, such as essential oils. Revenue streams for a floral infusion business can vary depending on the product offerings, customer base, and distribution channels. Some potential revenue streams for a floral infusion business and retail sales floral-infused beverages (like teas, syrups, and tinctures) directly to customers in physical stores, pop-up shops, or online platforms. A cost structure for a floral infusion business refers to the various expenses and costs involved in producing and selling. Floral Infusion offers a sustainable and innovative solution to the fragrance industry and biotechnology.

Keywords: *Floral infusion, Fragrance industry, Biotechnology, Sustainability, Revenue stream.*

PP-06

Interaction Analysis of *Ficus religiosa* Plant with the Begomovirus

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ABSTRACT

Begomovirus is classified as a plant pathogenic virus belonging to *Geminiviridae* family. It primarily infects dicotyledonous plants. These viruses are transmitted by *Bemisia tabaci,* which serves as a vector. These viruses cause huge economic loss by the complete destruction of important cash crops. To overcome the phytopathogenicity of Begomovirus, several antiviral sprays and insecticides have been made, but these sprays and insecticides prove to have more harmful effects than benefits. The accumulation of such antiviral sprays and insecticides occurs in plant or crop tissues, posing a direct threat to human life by disrupting the food chain. Instead of synthetic chemicals and antivirals, alternative harmless phytochemicals can be made that are free of hazardous byproducts, eco-friendly, non-toxic, and easy-to-use. These alternative phytochemicals can compete with viruses. *Ficus religiosa* has been extensively reported to have antiviral properties among various trees and plants. Thirteen phytochemicals from *F.virens* and seventy-nine from *F.religiosa* were selected for study. These plant parts, including barks, roots, and leaves, contain bioactive compounds that are highly effective against viruses, with antioxidant properties. PubChem was used to retrieve chemical structures of phytochemicals and opened through chimera. It was minimized and saved as a mol2 format. To visualize the best viral inhibitor, molecular docking analysis of phytochemicals with targeted proteins was performed, and for analysis of free binding energies, potential inhibitors and receptors were done. Ligand molecules with targeted proteins that showed the most appropriate interactions were chosen based on their score. These were then selected as potential antiviral drugs. For potential drug development, amino acid interactions and binding energies were determined. Results showed that bioactive compounds of *F.religiosa* act as promising antiviral drug candidates that can be further evaluated. Among seventy-nine phytochemicals, Alpha-Amyrenyl acetate, Tannin, and Lupen 3 one showed higher binding energies with the virus, so they could inactivate Begomovirus.

Keywords: *Begomovirus, Phytochemicals, Bioactive compounds, Antiviral sprays, Binding energy, Drug development.*

PP-07

Phytochemical Profiling and Biological Assessment of Three Plant Species

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ABSTRACT

The medical world is divided into two groups preferring herbal or orthodox medicines to treat different ailments; however, herbal medicines are proven to be highly therapeutic, moderate mechanism of action and are perceived to be safe with long-term activity. This notion has rationalized with the objective of the current study to elucidate the therapeutic potential of *Fagonia cretica*, *Prunus persica*, and *Momordica charantia* in n-hexane and acetone fractions at different concentrations by performing the anti-hemolytic, anti-inflammatory, thrombolytic, anti-oxidant, anti-diabetic, and DNA damage assay. In the evaluation of thrombolytic and anti-diabetic activity, *Prunus persica* revealed superior potential among all fractions, while *Fagonia cretica* fractions showed the highest anti-hemolytic activity with percentages of 95% at a certain concentration, anti-inflammatory as well as antioxidant activity. The extracts of *Prunus persica* and *Momordica charantia* possess the highest anti-thrombolytic potential i.e. 80% in peach and 67% in *Momordica Charantia,* being efficient in medicinal applications. These activities of plant extracts contribute to the presence of bioactive phytochemical constituents, mainly polyphenolic components. The current study provides experimental evidence that supports the use of natural products leading to therapeutic and pharmacological credence to herbal medications.

Keywords: *Phytochemical, Medicinal, Momordica charantia, Prunus persica, Fagonia cretica, anti-diabetic, anti-hemolytic, anti-oxidant, anti-inflammatory, thrombolytic*

PP-08

Genome-Wide Identification and Expression Analysis of Kinase Proteins in Land Plants, with a Focus on *Gossypium hirsutum* under Abiotic Stress

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ABSTRACT

The kinase proteins are a superfamily of plants and are involved in diverse biological and molecular functions for better adaptation of land plants. The current study aims to identify the kinase superfamily in land plants, covering from mosses to angiosperm. We have identified 49,611 genes in 32 land plants

across mosses, bryophytes, lycophytes, gymnosperms, and angiosperms. All identified genes were classified into 26 major classes (I-XXVI) based on domain architectures e.g Proline kinase like Tyrosine (PkT), Leucine Rich Repeat Receptor Kinases (LRR), Leucine Rich Repeat Receptor-Proline like Kinases (LRR-Pk), Leucine Rich Repeat Receptor-Proline like Tyrosine Kinases (LRR-PkT), and Proline kinase (PK) and "X" represents other than listed domains. Comparative genomics revealed evolutionary history, duplication, divergence, gene gain/loss, species relationships, and structural diversity of kinases in land plants, highlighting their molecular complexity and adaptive significance. Molecular docking analysis demonstrated the preferential binding of ATP to Gossypium hirsutum (*G. hirsutum*) kinase proteins, indicating their conserved phosphorylation mechanisms. Furthermore, SNP and Indel analyses in salt-resistant (Mac7) and salt-susceptible (Coker 312) cotton genotypes revealed genetic variations potentially linked to differential stress responses. Expression profiling highlighted strong upregulation of OG12_LR_GhPk08 in G. hirsutum under both biotic (e.g., Xanthomonas citri) and abiotic (e.g., salt, heat, and cold) stress, as well as in specific tissues like roots and seedlings. Conversely, consistently downregulated genes include OG7_LRR_GhPk11, which remained unresponsive across all biotic and abiotic conditions despite its presence in the genome, suggesting a limited role in stress responses. Moreover, the qRT-PCR based expression analysis of three genes (OG12_LR_GhPk08: Gohir.D08G10000, and OG35_Pk_GhPk02: Gohir.D05G155340) of Mac7 and Coker312 under salt stress demonstrated that these genes were highly expressed in both accessions under salt stress while OG44_PkT_GhPk09: Gohir.A03G148700 expression was decreased in both varieties under the same condition. The study provides a deep insight into the diversity and function of kinase genes in land plants, particularly cotton, and can be helpful in future research on kinome-related studies.

Keywords: *Kinase, Cotton, Abiotic Stress, Evolution, Expression, Domain, Molecular Docking*

PP-09

Detection of Alpha-1-antitrypsin (Serpina1) Single Nucleotide Polymorphism (rs750766974) in COPD patients at Gulab Devi Teaching Hospital

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is a progressive lung disorder and the fourth leading cause of death worldwide. It primarily includes chronic bronchitis and emphysema, which disrupts normal breathing by damaging the airways and alveoli, respectively. While genetic predisposition

contributes to approximately 1% of cases, environmental factors such as smoking and air pollution play a predominant role in disease development. A crucial genetic factor in the two-hit model of emphysema is Alpha 1-antitrypsin deficiency (AATD), which results from mutations in the SERPINA1 gene on chromosome 14q32. Alpha 1-antitrypsin (AAT) serves as a key protease inhibitor, regulating neutrophil elastase (NE) activity in the lungs and preventing excessive tissue damage. Mutations in SERPINA1 lead to diminished AAT levels, causing unchecked NE activity, persistent neutrophilic inflammation, and progressive lung tissue degradation, ultimately heightening the risk of COPD. Investigating the molecular mechanisms underlying AATD offers critical insights into the genetic basis of COPD, paving the way for targeted therapeutic approaches. Current treatments emphasize AAT augmentation therapy to restore protease-antiproteases balance, along with anti-inflammatory and regenerative strategies to reduce lung damage. Future advancements should focus on gene therapy and precision medicine to correct genetic deficiencies and enhance disease management. A deeper understanding of AATD in COPD pathogenesis could lead to innovative therapeutic interventions, improving patient outcomes and reducing the global burden of COPD-related mortality and morbidity.

Keywords: *COPD, SNP, Alpha 1-Antitrypsin, PCR, Restriction digestion*

PP-10

Assessment of Human Toll-like receptor 4 (TLR4) Asp299Gly (rs4986790) Polymorphism on Susceptibility of Coronary Artery Disease

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ABSTRACT

Coronary artery disease (CAD) is a leading cause of global mortality, primarily resulting from cholesterol accumulation in the coronary arteries, leading to plaque formation and atherosclerosis. While environmental factors contribute to disease progression, genetic components also play a crucial role. The Toll-Like Receptor 4 (TLR4) gene is one such factor, promoting macrophage transformation into foam cells upon exposure to oxidized low-density lipoprotein (oxLDL). Elevated TLR4 expression has been observed in circulating monocytes and coronary plaques of acute coronary syndrome (ACS) patients, with increased levels in ruptured atherosclerotic plaques suggesting its involvement in plaque instability. These findings highlight TLR4 as a potential genetic marker for CAD susceptibility. The TLR4 gene spans 27,333 base pairs and is located on chromosome 9q32-q33. This study aimed to determine whether the Asp299Gly polymorphism in TLR4 is associated with CAD risk. A case-control study was conducted, including healthy individuals and cardiac patients. Genomic

DNA was extracted from blood samples, and the ARMS-PCR technique was used to amplify the Asp299Gly polymorphism region, followed by gel electrophoresis confirmation and statistical analysis using SPSS software. Our results indicated no significant association between the Asp299Gly single nucleotide polymorphism (SNP) and CAD, suggesting that this specific genetic variant does not contribute to disease susceptibility. While TLR4 remains a key mediator of atherosclerosis, further research on other polymorphisms and genetic markers is essential to better understand its role in CAD pathogenesis.

Keywords: *CAD, TLR4-gene, Asp299Gly, rs4986790*

PP-11

Association of Leptin and Leptin Receptor Genes Polymorphism with Obesity and Insulin Resistance in Pakistani Population

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ABSTRACT

Obesity is an emerging public health problem around the globe. Current studies have explored the prevalence of obesity and have touched epidemic magnitudes worldwide. Current reports suggest that genetic influences play a vital role in the pathophysiology of obesity and its comorbidities. The current study was carried out to evaluate the role of leptin (LEP) and leptin receptor (LEPR) gene variants in the pathophysiology of insulin resistance in the obese and morbid obese Pakistani population. This study was carried out on 270 subjects (Normal control subjects -155, BMI below 25 kg/m^2 and obese-180, BMI above 30Kg/m^2 or 95th percentile). The age range was selected 20-55 years, all participants were investigated for G2548A and Q223R, variants of LEP and LEPR, genes respectively. Their association with metabolic, hormonal, and anthropometric traits was evaluated. All participants were screened for blood pressure, height, and weight, waist and hip circumferences were recorded, and BMI and waist and hip Ratios (WHR) were calculated. Fasting serum glucose, lipid profile, serum insulin, and serum leptin hormone were estimated by using a semi-automated chemistry analyzer and ELISA technique respectively while HOMA-IR was calculated using a formula. Genomic DNA was isolated from the anticoagulated blood, and genotyping all samples was done using polymerase chain reaction and restriction fragment length polymorphism technique (PCR-RFLP). Obtained data was analyzed by SPSS version 17 software. In this study, LEP gene (G2548A) polymorphism was observed to be linked with obesity and its comorbidities. G allele of LEP polymorphism were seen to have 2.23 folds more risk of obesity in (95% CI=1.07–4.63). G allele of LEP polymorphism was associated with elevated

body weight, BMI, WHR, serum insulin, HOMA-IR, and serum leptin levels. There were no significant alterations observed in genotype and allele frequencies of LEPR (Q223R) polymorphism in normal subjects and obese subjects. There was no association of (LEPR) polymorphism found with the metabolic traits and anthropometric outcomes related to IR and obesity. In conclusion, it is suggested that the LEP polymorphism is a very important predictor for elevated serum leptin and obesity traits; carriers of the A allele had raised leptin levels, FBG, and HOMA-IR independent of BMI.

PP-12

Association of rs141502002, rs505151, rs777300852 and rs28362277 of *PCSK9* Gene Polymorphisms with Coronary Artery Disease in Pakistani Population

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ABSTRACT

Coronary Artery Disease (CAD) is a leading cause of mortality worldwide. It was regulated by cholesterol deposition in coronary arteries, which formed a waxy material; plaque, which narrowed down the blood circulation in coronary arteries, was a major risk factor for atherosclerosis to instigate CAD among individuals. Along with environmental aspects, various genetic factors contribute to the menace of CAD. The *PCSK9* gene was one of the associated markers that play a key role in the regulation of cholesterol levels in the bloodstream, influencing susceptibility to CAD. The present study aimed to examine the association of four Single Nucleotide Polymorphisms (SNP's: rs141502002, rs505151, rs777300852, and rs28362277) of *PCSK9* with CAD in the Pakistani population. For this purpose, a case-control study was carried out on healthy controls and CAD patients, and the ARMS-PCR technique was applied to genotype our subjected population. Moreover, our data was statistically analyzed using SPSS software. In conclusion, the current study indicated that only one novel variant, rs777300852, was prevalent and had a significant ($P<0.05$) association with CAD in the sampled cohort. Additionally, statistical association analysis revealed that decreased High-Density lipid cholesterol (HDL-C) concentration may be a pivotal risk factor for CAD.

Keywords: *coronary artery disease, PCSK9 gene polymorphisms, polymerase chain reaction, lipid profiling analysis, genotyped variants.*

PP-13

Association of SNP rs16969968 of CHRNA5 Gene with COPD Patients at Gulab Devi Teaching Hospital

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ABSTRACT

COPD (Chronic Obstructive Pulmonary Disease) is a common and painful respiratory illness that presents a major healthcare challenge globally. New research indicates that genetic factors play a role in the susceptibility and development of COPD, in addition to cigarette smoking and environmental factors. Genetic differences can change a person's vulnerability to environmental hazards, impacting inflammatory pathways and a decline in lung function. Multiple genes are responsible for COPD, with CHRNA5 being one of them. The α5 gene contains the non-synonymous SNP rs16969968, resulting in the D398N amino acid alteration that is the main cause of nicotine dependence. The study uses a comprehensive collection of COPD patients (n=50) and controls (n=37) to identify and investigate the CHRNA5 gene polymorphism**.** It helps to investigate the molecular mechanisms by which CHRNA5 variations may affect disease pathophysiology, particularly their effects on pathways involved in oxidative stress, inflammation, and drug metabolism**.** The advanced molecular genetic tool ARMS PCR is used through which we amplified the specific SNP (rs16969968) by using allele-specific primers. Research indicates that the rs16969968 CHRNA5 gene variant demonstrates strong links to COPD and its relationship to chronic inflammation and nicotine dependency, which expedite disease progression. The findings emphasize how genetic studies both advance the creation of personalized treatments and enhance the understanding of how genes and environmental factors contribute to COPD development. The implementation of genomic findings into clinical practice will enable better disease management through specific treatment plans while improving risk assessment accuracy. Better care and hope for COPD patients are expected as the result of advancing research.

Keywords: *COPD, CHRNA5 gene, rs16969968, Nicotine Addiction, Smoking Exposure, Single Nucleotide Polymorphism, Chronic Inflammation.*

PP-14

Evaluation of Oxidative Stress and Eryptosis Induced by Gemifloxacin and Moxifloxacin in Erythrocytes

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ABSTRACT

Eryptosis, a programmed erythrocyte death, involves cell shrinkage, membrane blebbing, and phosphatidylserine exposure, often triggered by oxidative stress or energy depletion. Fluoroquinolones like gemifloxacin and moxifloxacin can induce eryptosis and are commonly used for respiratory infections like pneumonia, bronchitis, chronic obstructive pulmonary disease, and sinusitis. To evaluate and compare the oxidative stress and eryptotic potential of gemifloxacin and moxifloxacin. This study was conducted at the University of Agriculture, Faisalabad. Blood samples were collected, and after erythrocytes isolation via centrifugation, cells were treated with therapeutic doses of gemifloxacin (3 µM, 6 µM) and moxifloxacin (3 µM, 6 µM) and incubated for 48 hours at 37°C. Oxidative stress was measured by the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (POD) using enzyme-linked immunosorbent assay (ELISA). Hemolysis percentage and mean corpuscular volume (MCV) were recorded to see necrotic and eryptotic effects. EGTA (ethylene glycol tetraacetic acid) was used to chelate calcium and determine its role in eryptosis. Statistical analysis was performed using ANOVA and Tukey's test. Both fluoroquinolones induced oxidative stress, causing dose-dependent decline in SOD, CAT, and POD activities. Increased hemolysis and cell shrinkage confirmed eryptotic effects, marked by phosphatidylserine translocation and membrane blebbing. EGTA chelation reduced these effects, highlighting the role of Ca^{2+} influx. Gemifloxacin and moxifloxacin induce oxidative stress and eryptosis in erythrocytes by compromising antioxidant defense mechanisms and increasing free radical production. This study highlights the potential cytotoxic effects of fluoroquinolones on erythrocytes, suggesting clinical implications for the patients receiving these antibiotics.

Keywords: *Eryptosis, oxidative stress, fluoroquinolones, gemifloxacin, moxifloxacin, red blood cells, Ca^{2+} signaling.*

PP-15

PCR-Based Diagnosis for Salmonella Typhi in Clinical Specimens

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ABSTRACT

Salmonella enterica serovar typhi (S. typhi) are Gram-negative bacteria that cause typhoid fever. Food or water contaminated with an infectious dose of S. typhi is a common cause of typhoid fever. Transmission may occur through poor hygiene and sewage contamination of the water supply. The source of infection may disseminate from an infected person (carrier) to healthy individuals by fecal sample contamination to drinking water or food consumables. Typhoid fever can be fatal in children and aged persons if it remains undiagnosed and untreated. The conventional diagnosis test for Salmonella infections involves the use of IgG and IgM antibodies but now this test is considered obsolete due to false positive Salmonella detection. Blood culturing is the only option available for the diagnosis of S. typhi infections, but it takes a long reporting time (3-8 days). For the early detection of typhoid, certain molecular tests of S. typhi could be used, including PCR-based detection methods. Compared to other blood culturing and microbiological testing, the overall diagnosis took less time, greater sensitivity and precise results. This study includes the screening and diagnosis of more than 300 blood samples from suspected Typhoid patients using PCR methods. PCR allows selective amplification to distinguish S. typhi from the other closely related species. The use of PCR can help to attain more sensitivity and specificity to detect microbial pathogens in clinical specimens. This study helps to provide a way towards S. typhi diagnosis using PCR blood samples.

Keywords: *S typhi; typhoid; AMR; drug resistance; XDR*

PP-16

Impact of Shisha, Cigarettes, and E-Cigarettes on the Lungs of Youngsters

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ABSTRACT

The trend of smoking in young people has become a standard part of our modern society. According to the World Health Organization (WHO), Southeast Asian countries have the highest rates of tobacco smoking. Prevalence of 47% in both genders of age 15 years and above have been reported by (WHO). Water pipe smoking, known by a variety of names like Shisha, Narghile, Ghoza, Hubble Bubble, and e-cigarette, has been in vogue for the last many centuries. Its origin from one historical account suggested that it was invented in India by a physician, Hakim Abul Fath, during the reign of Emperor Akbar as a less harmful method of tobacco use.1 Some suggested that it was first used in South Africa, Persia, Ethiopia, and other countries. It has been claimed that more than 100 million people worldwide smoke water pipes. It has been a common practice in the Arabian Peninsula, Turkey, India, Pakistan, Bangladesh, and China. As the addiction to shisha and cigarette smoking is increasing day by day among youngsters, especially students, we collect data from smokers and non-smokers to know the effect of shisha and cigarette smoking on the lungs and how it affects lung function. This study provides robust evidence of the detrimental effects of smoking on lung function, with significant reductions in FEV1, FVC, and the FEV1/FVC ratio among smokers compared to non-smokers. These findings are consistent with previous research and highlight the need for continued efforts in smoking prevention and cessation to improve respiratory health and reduce the burden of smoking-related diseases. Public health initiatives, policy measures, and further research are essential to address the challenges posed by smoking and protect population health.

Keywords: *FEV1, FVC, Smoking, lungs.*

PP-17

Pharmacological Management of Pituitary Macroadenoma: A Non-Surgical Approach to Tumor Control and Hormones Regulation

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ABSTRACT

Pituitary macroadenoma (≥10 mm) is a benign tumor arising from anterior pituitary glands that leads to significant endocrine dysfunction and mass effects. Traditionally, it is treated with transsphenoidal surgery, which carries significant risks like visual disturbances, hypopituitarism, and vascular complications, which may reduce the quality of life. The motivation for this study arises from firsthand observations of the challenges faced by patients undergoing treatment of pituitary macroadenoma. This experience has provided me with valuable insights to explore pharmacological alternatives that could

reduce the dependence on surgery and can control tumor growth and restore hormonal balance without the risk associated with surgery. By evaluating the effectiveness and limitations of these therapies, we aim to highlight their significance in improving patient outcomes while minimizing complications. This study is based on a narrative review of literature, including review articles and research papers. Additionally, based on personal observations, experience, and conceptual reasoning, these findings provide an overview of the potential of non-surgical treatment strategies while minimizing patient risk. It was seen that pituitary adenomas sometimes may not be cured by surgery and cause severe problems. To avoid this, more effective drugs were used for the management of pituitary tumors that shrink the tumor size and help restore hormonal balance. The observations showed that many patients were successfully treated using pharmacological management without any risk and experienced an improved quality of life. Overall, we can conclude that pharmacological management presents a promising, non-surgical alternative for controlling pituitary macroadenoma. Moreover, it offers significant benefits such as reduced complications, improved quality of life, and suitability for high-risk patients. However, further research and clinical trials are needed to optimize treatment protocols and explore potential combination therapies for better patient outcomes.

Keywords: *Pituitary macroadenoma, pharmacological management, hormone regulation, Tumor control, Patient outcomes*

PP-18

Human DNA Isolation and Characterization from Bedbugs *(Cimex lactolarius)*

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ABSTRACT

Bedbug *Cimex lectolarius* is a Hematophagous ectoparasite that feeds on the blood of various vertebrate host including human. Human DNA can be extracted, and a host DNA profile can be generated from un-degraded human DNA extracted from small volume of the ingested human blood isolated from bedbugs in PBM (post blood meal) stage for individual identification. For human DNA extraction and characterization, bedbugs were collected manually from different residential areas and were preserved in absolute ethanol on -20 degrees. The human DNA was extracted from those bedbugs by manual organic PCI method. Human mitochondrial control region and HVR2 were amplified to confirm the isolation of mtDNA. Gender of the host of bedbugs were then determined using AmelT and HumAmel primers. Lastly the isolated human DNA were amplified by all the six common eye color primers (HERC2, TYR, OCA2, IRF, SLC24 and SLC45) followed by human eye color SNPs

profiling. The human DNA was specifically extracted from a single bedbug through manual PCI method, human mtDNA was amplified from the bedbugs followed by the human host gender determination and lastly the genes responsible for human eye color were amplified and some of the SNPs that regulate human eye color were also amplified. However, the SNPs results were not good enough to predict the eye color of the human host through IrisPlex online webtool.

Keywords: *Cimex lectolarius, Hematophagous ectoparasite, human DNA, mtDNA*

PP-19

Optimization of Autosomal STR Markers for Equine Genotyping Using Multiplex PCR

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ABSTRACT

The investigation of horse lineage was of paramount importance in the registration of different breeds, trade, and formulation of studbooks. The pioneering technique of DNA fingerprinting emerged as the first highly responsive method reliant on DNA for individual identification and the examination of genetic affiliations. Microsatellites were a valuable tool for analyzing the genetic variations present among different horse breeds. The International Society for Animal Genetics (ISAG) has endorsed a set of 17 specific Short Tandem Repeats (STRs) for equine identification, although these can be quite expensive to obtain through commercially available multiplex kits. To determine whether five autosomal STR markers (HMS6, HMS7, ASB23, VHL20, and LEX14) were optimized using multiplex PCR for equine genotyping. DNA was extracted from a Thoroughbred horse blood sample via an organic extraction method. Sensitivity analysis determined the optimal PCR concentration. Genotyping was performed on the ABI PRISM® 3100XL, and data was processed with Gene Mapper ID 3.2v software. The optimal conditions for multiplex PCR of HMS6, HMS7, ASB23, VHL20, and LEX14 primers were 60°C annealing temperature, 3ng DNA concentration and 6µM primer concentration. A 12.5µL PCR reaction volume was recommended for cost efficiency. The results of this research have the potential to create a cost-effective, regionally produced multiplex PCR kit. This kit would be designed for analyzing parentage lineage within the Equine family in Pakistan, incorporating ISAG-recommended markers: VHL20, HMS6, HMS7, ASB23, and additionally LEX14. It could significantly streamline the import and export of horses in Pakistan.

Keywords: *Microsatellites, Polymorphism, Genotyping, Capillary Electrophoresis.*

PP-21

Analysis of Hotspot Mutations of the Ltbp2 Gene: A Predominant Driver of Primary Congenital Glaucoma

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ABSTRACT

Primary Congenital Glaucoma (PCG) is an inherited eye condition that affects the anterior chamber angle of the eye and the optic nerve head. This study aimed to identify genetic variants associated with PCG in the Pakistani population and gain insights into the role of LTBP2 in causing this condition. Thirty-seven samples were selected out of fifty affected individuals, and genomic DNA was extracted using the phenol-chloroform method. Specific primers were designed to amplify exons 1 and 4 of LTBP2 using polymerase chain reaction (PCR). Sequencing was performed using the fluorescence-based chain terminator (dye-deoxy) method. Two mutations, p.Q111X and p.R299X, were identified in a significant proportion of patients with PCG. p.Q111X was found in 27.6% of cases, and p.R299X was present in 42.5%. Age of onset, familial history, and type of blindness (bilateral or unilateral) were linked to the mutation types. This study highlights the importance of early and accurate diagnosis of PCG to initiate appropriate management before irreversible damage to vision occurs. The genetic information obtained from this study could facilitate genetic counselling and targeted molecular prognosis, including prenatal screening, for families at risk of PCG recurrence. This study sheds light on the genetic basis of PCG in the Pakistani population, emphasizing the significance of genetic counselling and early intervention to effectively manage this visually impaired condition.

Keywords: *Primary Congenital Glaucoma, LTBP2, Genetic Variants, Pakistan, Genetic Counselling, Early Intervention.*

PP-22

Pharmacogenetic Perspective on Clopidogrel: ABCB1 C3435T and CYP2C19*2 Polymorphism in Swat Population

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ABSTRACT

A key component of the prevention of coronary artery disorders is antiplatelet treatment. Among the various antiplatelet medications often thought to be used unrestrictedly in individuals with peripheral vascular disorders is clopidogrel. In individuals with acute coronary syndrome, it can lower mortality

and improve cardiovascular outcomes without making bleeding more likely. The *ABCB1* gene is responsible for producing P-glycoprotein, an intestinal transporter that significantly influences clopidogrel absorption. Furthermore, genetic variations in cytochrome P4502C19 (*CYP2C19*), the primary enzyme in charge of metabolizing clopidogrel, may be the cause of notable variations in antiplatelet efficacy. This study aimed to examine how genetic variations in the *ABCB1* and *CYP2C19* genes affect clopidogrel's effectiveness. Blood samples were collected from patients taking clopidogrel tablets at different hospitals in Swat district, including Saidu teaching hospital, Kabal, Matta, and Khwazakhela. Further, we focused on patients aged 25-60. The DNA from blood samples was extracted by the PCIA method and amplified on C3435T and CYP2C19*2 primers. There were three *CYP2C19*2* genotypes: three represented homozygous normal metabolisms of clopidogrel (1*/1*), good metabolizer of clopidogrel, thirteen had heterozygous metabolism (1*/2*) intermediate metabolizers, and eleven had homozygous mutant metabolisms of clopidogrel with decreased effectiveness of the medication. We also identified three *ABCB1 C3435T* genotypes: one individual homozygous mutant (TT), which is associated with decreased transporter function and potential alterations in drug bioavailability; three individuals were heterozygous (CT), which indicate partially altered transporter activity; and seven were wild type (CC), which indicates normal drug absorption.

Keywords: *ABCB1 C3435T, CYP2C19*2, Polymorphism, Clopidogrel, Swat population*

PP-23

Synthesis of anti-Interferon Beta 1a Antibody in Mice and its Characterization

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ABSTRACT

Multiple Sclerosis (MS) is a disease of the nervous system. In this autoimmune disease, the immune system of the body starts attacking the healthy nerves and degenerating the myelin sheath present on them. This results in the formation of scars on the multiple sites on nerves. In patients of MS, brain-to-body communication is affected and other symptoms like the decline in mobility, blurriness and double vision, Lhermitte sign, and lack of sensation are noticed. Different therapies for multiple sclerosis are used but the most used therapy is disease-modifying therapy using beta interferons. Considerable clinical problems are the formation of anti-IFN neutralizing antibodies (NABs) against the drug, which decreases the therapeutic effectiveness. To confirm this, we immunized the mice with BSA and OAS-conjugated activated interferon beta 1a for five weeks, then we took the blood from the mice through cardiac puncture and isolated the serum by centrifugation. We performed ELISA and Immuno Dot Blot tests, both of which showed positive test results which confirmed the formation of

anti-IFN neutralizing antibody. In future we can further characterize these NABs to exactly know about the mechanism of antibody production against the injected drug, to launch an appropriate treatment mechanism where clinical efficacy of the treatment would sustain.

PP-24

Optimization of Submerged Fermentation Conditions for Lipase Production from *Bacillus mycoides* through Response Surface Methodology

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ABSTRACT

Lipases or triacylglycerol hydrolases (EC 3.1.1.3) are hydrolases that break down the carboxylic ester linkages in acylglycerols to release glycerol and fatty acids. Lipases are highly desirable for a variety of industrial applications, including food, detergents, pharmaceuticals, textiles, cosmetics, leather, and paper. The main objective of the current research work was to maximize lipase production from *Bacillus mycoides* through response surface methodology. Firstly, the lipase activity of *B. mycoides* was determined on nutrient agar plates containing phenol red indicator and 2 % each substrate (Tween 80, sunflower, olive, canola, soybean, and almond oils). Apart from sunflower oil, bacterial growth was observed in the presence of all substrates. Secondly, *B. mycoides* was allowed to grow in nutrient broths containing 2 % of each substrate. After 24 h of incubation at 37 °C, cultural supernatants were utilized in lipase assays. Reaction mixture consisted of 2 ml supernatant, 2 ml 1.5 M Tris-HCl (pH 9.0), 0.05 ml Tween 80- and 0.1-ml phenol red. The reaction mixture was incubated at 55 °C for 20 minutes followed by detecting the production of oleic acid from tween 80 spectrophotometrically at 450 nm. Highest lipase activity was detected in the cultural supernatant obtained by growing *B. mycoides* in the presence of tween 80. Thereafter, statistical Box Behnken design (Design-Expert 13) was used for enhancing lipase production under submerged fermentation conditions based on the interaction of three parameters: A (Fermentation time), B (Temperature), and C (Tween 80 as a lipase substrate). The maximum lipase activity was detected in a cultural supernatant that was obtained by growing *B. mycoides* in nutrient broth (containing 5 % tween 80) for 24 h at 37 °C. Activity at high temperature and pH make lipase from *B. mycoides* an ideal candidate for the detergent, pharmaceutical, and cosmetics industry.

Keywords: *Lipase, Bacillus mycoides, Box Behnken design, optimized production*

PP-25

Application of Response Surface Methodology for Optimizing the Extracellular Conditions for Protease Production from *Bacillus mycoides*

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ABSTRACT

Proteases (EC 3.4.21) are commercially important enzymes with a market size of USD 3.73 billion (2025). Alkaline proteases are the most significant among proteases for industrial applications (detergent additives in laundry applications). Alkaline protease either requires metal ions for catalysis (metalloprotease) or has a serine core (Asp-His-Ser). Commercial proteases are primarily derived from bacteria, especially the *Bacillus* group, which are more active and vibrant extracellular alkaline protease producers in the industrial sector due to their high yield and ease of culturing. The current research work aims at the application of response surface methodology for maximizing the production of alkaline protease from *Bacillus mycoides* under submerged fermentation conditions. Statistical Box Behnken design (Design-Expert 13) was used for maximizing protease production from *B. mycoides* based upon the interactions of three variables: A (Fermentation time), B (pH) and C (casein percentage). Protease was produced under different cultural conditions, and cultural supernatants were analyzed for casein (1 %) hydrolysis through agar well diffusion assay (plates were incubated at 37 °C for 24 hours). Halo zones formed around the wells containing cultural supernatants were measured. The highest zone of casein hydrolysis (11.5 mm) was observed with the cultural supernatant obtained by growing *B. mycoides* in the liquid medium (pH 8.0) containing 1 % casein for 24 h at 37 °C. Hence, the Box Behnken design was quite helpful in enhancing the production of protease from bacterial species like *B. mycoides*. Purified alkaline protease produced from *B. mycoides* may serve as a valuable additive in detergents.

Keywords: *Alkaline protease, Bacillus mycoides, Box Behnken design, optimized production, detergent applications*.

PP-26

Production, Purification and Comparative Insights on Novel Phytases from Different Bacterial Strains

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ABSTRACT

Enzymes play an important role in various industries due to their ability to catalyze biochemical reactions with remarkable efficiency, specificity, cost-effectiveness and eco-friendly nature. Advancements in enzyme engineering have led to the development of tailored enzymes with enhanced stability and activity. Phytase is a vital enzyme in biotechnology as a feed additive for non-ruminants. It reduces phytate content in fodder, enhances phosphorus and mineral bioavailability, lowers feed costs, and minimizes environmental pollution. Phytases have a major role in enhancing nutrient absorption, improving animal growth performance, and reducing the dependency on inorganic phosphate supplements. This research focuses on optimizing phytase efficiency to maximize its benefits in livestock and aquaculture industries. Specifically, this study focuses on assessing the phytases from different organisms. The phytase genes from *Bacillus subtilis* R5 and Escherichia coli were cloned and expressed in E. coli BL21-codon Plus (DE3)-RIL. The purified phytase from E. coli was purified as inclusion bodies, while *B. subtilis* R5 Phytase was a soluble protein. The phytase activity for these enzymes was compared through comprehensive biochemical testing. Stability and functionality across diverse conditions, including temperature, pH, substrate concentration, and other kinetic parameters, were also checked.

Keywords: *Phytase; Catalytic efficiency; Cost-effectiveness; Mineral bioavailability*

PP-27

Production and Biochemical Characterization of a Recombinant Catalase from *Clostridium difficile*

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ABSTRACT

Catalases (EC 1.11.1.6) are oxidoreductase enzymes in living organisms that are crucial for getting rid of hydrogen peroxide from the cell, which is a harmful byproduct of cell metabolism that needs to be

neutralized. This study aims to clone a manganese catalase from the mesophilic bacterium *Clostridium difficile*. Heterologous expression was obtained for the corresponding gene, Cat Cd, in Escherichia coli. The recombinant protein is majorly produced in the soluble form and purified using Ni-NTA chromatography. To characterize this enzyme for its potential application in industry, optimum pH, temperature stability, kinetic parameters, and cofactor specificity were determined. Additionally, a comparative analysis was made with other known catalases to assess differences in stability, catalytic efficiency, and structural features that make it a promising candidate for applications in food processing, pharmaceutical development and environmental bioremediation.

Keywords: *Clostridium Difficile; Manganese Catalase; Cloning and Expression; Hydrogen peroxide*

PP-28

Assessment of Banana Peels as Substrate for the Production of Gallic Acid via Solid State Fermentation Using Response Surface Methodology

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ABSTRACT

As a secondary metabolite, gallic acid is a significant organic acid that is present in various plant components. Around the world, there is an increasing demand for gallic acid. These days, tannase, which hydrolyses tannin into glucose and gallic acid, is produced by microorganisms such as bacteria, fungus, and yeast. It also demonstrates antibacterial, antifungal, and anticancer qualities. A wide variety of plant components, including bark, fruit seeds, fruit peels, leaves, and galls, contain tannin. Because tannin and gallic acid share a similar structure, tannin is the ideal substrate for gallic acid. Current research intends to use solid-state fermentation to produce gallic acid from tannin-rich byproducts. Temperature, inoculum size, pH, and moisture content were among the many bioprocess parameters optimized using the central composite design (CCD) of the response surface methodology. The *Musa paradisiaca* linn peels were used as the substrate, and after 72 hours of incubation, the maximum amount of gallic acid (2.62 mg/g) was produced at a moisture content of 60%, inoculum size of 2 mL, pH 5, and temperature of 35 ºC. The importance of the proposed model was shown by the variance analysis, which produced an F-value of 12.940 and a p-value of 0.000. The gallic acid produced was validated using FTIR. HPLC analysis confirmed the purity of gallic acid. According to the results, microbial conversion of fruit peels high in tannic acid to gallic acid is a feasible way to produce gallic acid in large numbers for commercial application.

Keywords: *Tannase, tannic acid, gallic acid, banana peels, solid-state fermentation, response surface methodology*

PP-29

Purification and Refolding of The SUMO Fused Knob Domain of Fowl Adenovrus-4

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ABSTRACT

The prevalence of fowl adenovirus-4 (FAdV-4) infections such as inclusion-body hepatitis hydropericardium syndrome (IBH-HPS), poses a significant threat to the global poultry industry, including Pakistan, which ranked as the 11th largest poultry producer worldwide. The poultry sector contributes approximately 1.5% to the GDP of Pakistan. IBH-HPS causes 80% mortality in the poultry industry, leading to substantial economic losses and adverse effects on poultry trade in affected regions. To overcome this loss, efforts are required, such as the production of fourth-generation recombinant subunit vaccines targeting IBH-HPS. Research indicates that the structural protein, fiber-2 of FAdV-4 has a knob domain that exhibits remarkable immunogenic properties and is a promising vaccine candidate against IBH-HPS. However, a significant drawback hindering the use of this prokaryotic expressed recombinant protein is the production of the protein in the insoluble form as inclusion bodies. Hence, study was designed to use the SUMO (Small Ubiquitin like Modifier) tag to get the soluble expression of the recombinant protein. For this purpose, Fiber-2-Knob domain was cloned into the vector pE-SUMOproKan. Recombinant protein was expressed in BL21 (DE3) strain of E. coli in the presence of 0.25 mM IPTG as an inducer. Recombinant protein was expressed as inclusion bodies both at 37 °C and 16 °C. The insoluble recombinant protein was solubilized in 8 M urea and subsequently purified by nickel affinity chromatography under denaturing conditions. To achieve the protein in its native form, the protein was refolded through a step-wise gradient dialysis method. The protein remained in the soluble fraction resulting in the refolding of the protein upon complete removal of the denaturant. Post-dialysis of the recombinant protein was quantified by Bradford assay which estimated the yield of the protein to be 8.44 mg/L of culture volume. Further, native polyacrylamide gel electrophoresis analysis revealed that the protein exists in various oligomeric forms. It is anticipated that the purified refolded knob domain can be further utilized for structural studies of fiber-2-knob domain using advanced techniques and for immunization trials in chickens to combat FAdV-4 infections.

Keywords: *Recombinant protein expression, Protein purification, SUMO tag, Fowl Adenovirus-4, Vaccine development*

PP-30

Molecular Cloning, Expression & Purification of Encapsulin like Hypothetical Proteins from *Thermococcus kodakarensis*

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ABSTRACT

Encapsulin nanocompartments are self-assembling protein cages that enclose enzymes. They are dynamic with enormous applications in biomolecular storage, enzymatic catalysis, and synthetic biology. Although encapsulins are well characterized in bacteria, their occurrence and potentially functional roles in archaea are largely unknown. One example is the hyperthermophilic archaeon, *Thermococcus kodakarensis*, which encodes putative encapsulin-like proteins. They have distinct structural and functional characteristics that hold potential for biotechnology applications. The genes are generally associated with phage-related domains, suggesting potential evolutionary relationships and functions in biomolecular storage. In this study, we successfully cloned, expressed, and purified encapsulin-like proteins, namely *Encapsulin A* and *Encapsulin B* from *T. kodakarensis*. The genes coding for both the proteins were amplified, cloned into pColdI expression vector through restriction ligation method. Successful cloning of genes was confirmed by colony PCR, restriction digestion, and DNA sequencing. Expression trials in *Escherichia coli* BL21 (DE3), BL21CodonPlus, and Rosetta (DE3) strains at 37°C, 30°C and 20°C with different IPTG concentrations was carried out. The optimal expression of *Encapsulin A* and *Encapsulin* was achieved by inducing cultures at 30°C with 0.5 mM IPTG (overnight) in BL21(DE3) cells. The recombinant protein (~10 kDa) was purified using Ni-NTA affinity chromatography, appearing as a distinct band on SDS-PAGE. Analysis of protein solubility indicated that a considerable portion remained in the soluble fraction. Then purified protein appears to make oligomer on Native PAGE. Furthermore, the oligomer state of the *Encapsulin A* and *Encapsulin B* was also determined by size exclusion chromatography. These results add to our basic understanding of archaeal encapsulins and their potential applications in biotechnology, nanotechnology, biomolecular storage, and drug delivery.

PP-31

Characterization of Recombinant Human Serum Amyloid P-Component (SAP)

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ABSTRACT

SAP is a universal component of fibrils, which are aggregates of misfolded proteins. There is a limited understanding of the molecular interactions governing the binding of SAP with fibrils. The present study deals with the engineering and characterization of human SAP derivatives. Mature human *SAP* gene was sub-cloned in expression vectors pET-21a and pET-28a, giving *SAP (618 bp)* and *His-SAP* (678 bp) which were analyzed by double digestion using *Nde*I and *Hind*III, and confirmed by Sanger sequencing, followed by the expression in *Escherichia coli* Rosetta (DE3) pLysS. Both the constructs gave significant expression at 37 °C with 8-hr post-induction using 0.2 mM IPTG, yielding 130 – 160 OD_{280} inclusion bodies from 1 L of culture. The molecular weight of SAP and His-SAP was 23.4 and 25.5 kDa respectively. Since these proteins were expressed as inclusion bodies, 390 OD_{280} of SAP and 300 OD_{280} of His-SAP were subjected to *in vitro* refolding, which was monitored by DTNB assay for thiol estimation, followed by 15% Tricine PAGE. The results showed that both the SAP derivatives had faster mobility under non-reductive conditions, as compared to reductive, which could be attributed to their properly native globular structure. The refolded reconstitution was purified, characterized by circular dichroism spectroscopy and pull-down assays with different fibrils. CD spectroscopy showed 70% beta-sheets and 15% alpha-helical regions. Under *in vitro* physiological conditions, SAP derivatives showed significant binding with serum amyloid A and insulin fibrils in the presence of 2 mM calcium ions, even though these derivatives were non-glycosylated. The present study highlights that the recombinant SAP produced in *E. coli* exhibited similar universal characteristics as that of native SAP, i.e. binding affinity towards fibrils, which can be further investigated to study the fibrillation mechanisms involved in amyloidosis, autoimmune diseases, and several inflammatory diseases.

Keywords: *Serum amyloid P-component, Fibrils, Insulin, Pull-down assays, Amyloidosis, Autoimmune diseases.*

PP-32

Pcal_0606: A Hyperthermophilic Phosphoglucose/Phosphomannose Isomerase with Exceptional Stability and Activity

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ABSTRACT

Pyrobaculum calidifontis genome encodes homologs of all enzymes involved in the glycolytic pathway. Most enzymes of this pathway *have been characterized by our group*. However, the putative phosphoglucose/phosphomannose isomerase, encoded by the open reading frame *Pcal_0606*, remains to be fully characterized. *In silico* analysis showed multiple potential substrate-binding pockets at the dimeric interface of *Pcal_0606*. The gene encoding *Pcal_0606* was cloned and heterologously expressed in *Escherichia coli*, yielding a soluble recombinant enzyme. Kinetic characterization of Pcal_0606 showed that its optimum working temperature and pH were 90 °C and 8.5, respectively. This metal-independent enzyme, under optimal conditions, exhibited apparent K_m values of 0.33, 0.34, and 0.29 mM for glucose 6-phosphate, mannose 6-phosphate, and fructose 6-phosphate, respectively, with corresponding V_{max} values of 290, 235, and 240 µmol min^{-1} mg^{-1}. These results suggested that the enzyme catalyzes the reversible isomerization of these substrates with comparable catalytic efficiency. Notably, *Pcal_0606* displayed exceptional thermostability, retaining activity with a half-life of ~50 min at 100 °C. To the best of our knowledge, *Pcal_0606* represents the most active and thermostable bifunctional phosphoglucose/phosphomannose isomerase characterized to date.

Keywords*: Glycolytic pathway; Thermostability; Bifunctional; Catalytic efficiency*

PP-33

Optimization of Culture Conditions for Production of Antibiotics by Actinomycetes

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ABSTRACT

Actinomycetes are renowned for natural antibiotics production. The study objective was to optimize and assess the antimicrobial properties of soil actinomycetes. By using serial dilution and isolation method a total of 40 bacterial isolates were isolated from ten soil samples. The actinomycetes were

identified by colony characterization, microscopy, and biochemical tests. Out of 40 isolates obtained from the 10 soil samples, 15 were identified as actinomycetes. Fifteen actinomycetes were subjected to the production of antibiotics in a liquid fermentation medium. Initial screening was done by using the agar well diffusion method. *Escherichia coli*, and *Bacillus subtilis* were used as test organisms during the antibiotics screening process. After the screening process, A5 was selected because the highest zone of inhibition was observed in the presence of antimicrobial substances produced by this A5 isolate. A maximum 15 mm zone of inhibition was observed by using A5 isolate. This strain was further selected for optimization studies. To determine the optimal culture conditions, various physicochemical parameters were tested, including carbon source, nitrogen source, temperature, pH, incubation period, and agitation rates. After optimization, a maximum of a 22 mm zone of inhibition was observed by antibiotics produced in the fermentation medium by the A5 Actinomycete strain. It proves that optimization enhances the amount of antibiotics produced by actinomycetes.

Keywords: *Submerged Fermentation, Culture medium, Actinomycetes, Antibiotics.*

PP-34

Molecular Cloning, Expression Optimization, and Purification of a Glycerophosphodiesterase from a Hyperthermophilic Archaeon *Pyrococcus abyssi*

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ABSTRACT

Glycerophosphodiesterases (GDPDs) are ubiquitous and found in three domains of life (bacteria, archaea and eukarya). They are involved in phospholipid metabolism by catalyzing the hydrolysis of glycerophosphodiesters into glycerol 3-phosphate (G-3-P) and corresponding alcoholic moieties. Additionally, GDPDs serve as important contributing factors to the virulence of various pathogenic bacteria and fungi. Recently, they have acquired much attention in the field of bioremediation owing to their ability to hydrolyze organophosphate (OP) based pesticides and nerve agents. The purpose of the current research work was the heterologous production of a Pa-GDPD from *Pyrococcus abyssi* in *E. coli* under optimized conditions followed by its purification. Pa-GDPD gene (~768 bp) was acquired from genomic DNA of *P. abyssi* through PCR amplification followed by its cloning in pJET1.2/blunt and pET-28a vectors. Pa-GDPD gene expression was higher in *E. coli* Rosetta (DE3) pLysS in comparison to *E. coli* BL21 CodonPlus (DE3)-RIL. In addition, various inducer and extracellular cultural conditions were optimized for obtaining high levels of expression of Pa-GDPD. Expression of the target gene was induced in M9NG medium (supplemented with 3 % ethanol) by using 0.6 mM

IPTG (isopropyl β-D-thiogalactopyranoside) at 37 °C for 8 h post-induction duration. Soluble recombinant enzyme (~30 kDa) was purified to homogeneity by using the combination of heat-treatment (70 °C for 30 min) and Ni-NTA (nickel-nitrilotriacetic acid) chromatography. Pa-GDPD was eluted from Ni-NTA column by using 150-250 mM imidazole gradient. Biochemical characteristics of the purified recombinant Pa-GDPD need to be determined in order to explore its full potential for bioremediation of OP compounds.

Keywords: *Glycerophosphodiesterase, Pyrococcus abyssi, molecular cloning, expression optimization, purification.*

PP-35

Mining and Characterization of GTF1 from Locally Isolated Novel Species, *Apilactobacillus waqarii*

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ABSTRACT

Glucosyltransferases (GTFs) belong to the glycoside hydrolase (GH) family 70 are promising enzymatic tools for the synthesis of exopolysaccharides from renewable sucrose and starch substrates, a reaction that is widely seen in lactic acid bacteria (LAB). Depending on the specificity of GTFs, a large diversity of glucans containing variety of glucosidic linkages, like α-(1-2), α-(1-3), α-(1-4) and α-(1-6) are produced with varying degrees of branching, size, structure and spatial arrangement. Products of GTFs have great commercial value as food supplements and medical materials; therefore, these enzymes have attracted much attention from both science and industry. In the present study, computational tools were employed to identify GTF-encoding genes within the genome of *Apilactobacillus waqarii*, a novel LAB strain isolated locally. Three genes, designated GTF1, GTF2, and GTF3, were identified as being involved in exopolysaccharide synthesis. In silico characterization of GTF1 revealed four domains, including a catalytic domain containing conserved motifs and residues. 3D structure prediction and evaluation indicated disordered regions that were subsequently truncated to enhance protein stability. Primers were designed, and truncated GTF1 was cloned and recombinantly expressed in Escherichia coli. Overexpression of the recombinant proteins resulted in

the formation of inactive and insoluble inclusion bodies. Various refolding strategies were tested to reduce aggregation and promote correct protein folding; however, the protein remained inactive. GTF1, being the largest (235 KDa), was subjected to further truncations, including the disordered regions predicted by in silico analysis. Interestingly, a truncated version of the protein, retaining most of the wild-type residues (160 KDa), was found to be soluble and active when expressed in the bacterial system. This suggests that while the disordered region in the 3D structure was previously considered unfavorable, it contains residues essential for the solubility and activity of the protein.

PP-36

Developing Next-Generation L-Asparaginases in Tackling Therapeutic and Industrial Challenges

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ABSTRACT

L-asparaginases are vital in treating acute lymphocytic leukemia (ALL) and reducing acrylamide in food processing. However, commercially available enzymes from mesophilic sources face limitations such as glutaminase activity causing severe side effects, low in vivo stability requiring frequent dosing, and thermolability necessitating additional food processing steps. This study addresses these challenges by exploring hyperthermophilic L-asparaginase from *Thermococcus kodakarensis*, which is intrinsically thermostable but showed less activity at physiological conditions. Based on literature and structural data mutants were designed and compared with wild type. Engineered mutants L56D and T55Q-K299L demonstrated enhanced catalytic efficiency at physiological and optimal conditions, respectively. T55Q showed a >50% activity increase at 90°C for industrial use, while L56D exhibited a fourfold rise at 37°C, ideal for therapeutic applications. These enzymes were further investigated for their therapeutic potential on leukemic cell lines and acrylamide reduction in high-temperature cooking. Additionally, cost-effective production strategies were explored to reduce economic burdens. By overcoming the limitations of current enzymes, this study highlights the dual potential of hyperthermophilic L-asparaginases in advancing cancer therapy and sustainable industrial practices, aligning with global sustainability goals.

PP-37

Machine Learning-Powered Design and Optimization of a Novel Bifunctional Phytase-Protease Enzyme for Enhanced Phytic Acid and Protein Utilization

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ABSTRACT

The development of multifunctional enzymes through synthetic biology and artificial intelligence (AI) represents a cutting-edge approach to enhancing nutrient bioavailability in animal feed. In this study, a novel bifunctional phytase-protease enzyme was designed using advanced bioinformatics and AI-guided engineering strategies followed by machine learning as well. Phytase enhances phosphorus release from phytic acid, while protease improves protein digestibility, addressing two major nutritional bottlenecks in poultry nutrition. Functional domains were identified while disulfide bond formation was predicted. The novel enzyme construct was designed using machine learning-based linker optimization and motif generation to enable independent and stable domain activity. AI-driven prediction tools, including AlphaFold DeepMind, were employed for structural modeling and folding analysis. Optimal pH, thermostability, and solubility were predicted using EpHod, TemStaPro, and SoDoPE. Electrostatic and hydrophobic interaction mapping with Chimera and Pymol helped refine the interface region to prevent steric clashes. Docking simulations with AutoDock Vina confirmed the high binding affinity of phytase for phytic acid and protease for N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Molecular dynamics simulations using GROMACS validate structural stability and dynamic behavior under physiological conditions. The recombinant bifunctional phytase-protease construct will be synthesized and cloned into a vector and will be transformed into an *E. coli* strain to study enhanced protein expression and folding efficiency. Enzyme activity and stability will be assessed under varying pH and temperature conditions to evaluate functional performance. This AI-guided approach resulted in a stable and highly efficient bifunctional enzyme named Phy-Prot with enhanced nutrient degradation potential, paving the way for future applications in sustainable animal feed and industrial biocatalysis.

Keywords: *Protein engineering, Multifunctional enzyme, Phytase-protease, Machine learning, Structural optimization, Protein Expression.*

PP-38

Production of Recombinant DNA Polymerase I from *Geobacillus thermopakistaniensis* for Application in Isothermal Amplification Techniques

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ABSTRACT

The rapid rise of pandemics has emphasized the need for point-of-care diagnosis of causative agents to facilitate both treatment and the prevention of a major spread. The isothermal amplification techniques have been effective in rapid and accessible diagnostics and have become the focus in recent molecular diagnostic development. These techniques require thermostable DNA polymerases with strong strand displacement activities. This study involves the heterologous production of DNA polymerase I from the thermophilic microorganism *Geobacillus thermopakistaniensis*. The gene for DNA polymerase I (*polF*) was amplified and eventually cloned into expression vector pET-28a (+). Heterologous expression was achieved through induction in *Escherichia coli* Rosetta (DE3)-pLysS. The protein was purified through heat treatment, anion exchange chromatography, and hydrophobic exchange chromatography. The cloned gene consists of 2631 nucleotides encoding a protein of 876 amino acids with a molecular weight of 90 kDa and temperature stability up to 65°C. The multiple-sequence alignment showed that the protein exhibits high similarity to Pol I of family A DNA polymerase. Conserved domains for 5'-3' and 3'-5' exonuclease activities and 5'-3' polymerase activity were identified in the protein. The strand displacement activity has been determined. The polymerase activity of the enzyme was measured by polymerase activity assays using radiolabeled nucleotides. The enzyme was then explored for its application in isothermal amplification techniques based on its strand displacement activity. Isothermal amplification techniques amplify the genetic material at a constant temperature, therefore undermining the need for thermocyclers and trained personnel, making the diagnosis of infectious diseases efficient.

Keywords: *isothermal applications, recombinant DNA polymerase, diagnostic application.*

PP-39

Evaluation of *Bacillus spp.* as Direct-Fed Microbials for Poultry: Enzyme Production, Biofilm Formation, and Antimicrobial Activity

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ABSTRACT

The growing concern over antibiotic misuse as growth promoters and the rise of multidrug-resistant bacteria have led to restrictions on antibiotic use in livestock feed across several countries. Direct fed microbials (DFM) have emerged as promising alternatives, with *Bacillus spp*. being extensively studied due to their ability to form resilient endospores, produce antimicrobial compounds, and secrete various exogenous enzymes. This study aimed to evaluate and select *Bacillus* strains from environmental and poultry sources as potential DFM candidates based on their enzyme production, biofilm formation, and pathogen inhibition capabilities. A total of 31 *Bacillus* isolates were screened for amylase, protease, lipase, and phytase activity, identifying three superior enzyme-producing strains: *Bacillus subtilis* (1/3) and *Bacillus amyloliquefaciens* (2/3), confirmed through biochemical tests and 16S rRNA sequencing. Biofilm formation assays revealed strong biofilm synthesis in 11 out of 31 strains. Additionally, the isolates exhibited antimicrobial activity against *Salmonella enterica* serotype *Enteritidis* (26/31), *Escherichia coli* (28/31), and *Clostridioides difficile* (29/31). Previous in vitro and in vivo studies confirmed the resilience of selected strains under the gastrointestinal conditions of poultry. These findings suggest that *Bacillus*-based DFMs, with their diverse enzymatic and antimicrobial properties, could enhance poultry performance by improving nutrient digestibility, reducing intestinal viscosity, maintaining gut microbiota balance, and supporting intestinal health.

Keywords: *Bacillus spp., direct-fed microbials, poultry gut health, enzyme production, antimicrobial activity.*

PP-40

In House Production And Characterization of Plastic Degrading Enzymes

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ABSTRACT

Polyethylene terephthalate (PET) is commonly used in plastic bottles and disposable goods; however it is not biodegradable. Researchers are exploring enzymatic degradation as an eco-friendly solution, with significant interest in enzymes like PETases from Ideonella sakaiensis and LCC (Leaf-branch compost cutinase) for breaking down PET. PETase and LCC convert PET into mixture of monomers MHET (mono(2-hydroxyethyl) terephthalate), BHET (bis-2- hydroxyethyl terephthalate), TPA (Terephthalic acid) and ethylene glycol. To further degrade insoluble MHET and BHET into soluble monomers another accessory enzyme MHETase is also required. This study focuses on in-house

cloning, expression and purification of truncated LCC (leaf branch compost cutinase) gene to improve its efficiency in PET degradation. For this purpose, LCC was cloned in pCold and pET expressions vectors and expressed in various Escherichia coli expression strains i.e. BL21 (DE3), BL21-CodonPlus and Rosette (DE3). In order to optimize the expression, various inducer, time and temperature conditions were screened. However, the best expression was observed in BL21 (DE3) strain. Further, the LCC was purified using affinity chromatography. After successful expression and purification, the activity of this enzymes will be determined against the substrate PET (Polyethylene terephthalate).

PP-41

Deciphering the Role of Honey in Ocular Diseases by Integrating Network Pharmacology and Molecular Docking Approaches for Retinal Therapy

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ABSTRACT

Diabetic retinopathy (DR) and age-related macular degeneration (AMD) are the leading causes of vision loss globally. Conventional therapies for these conditions present challenges, prompting exploration of natural remedies like honey, which possesses anti-inflammatory, antioxidant, and antidiabetic properties. This study aims to explore the therapeutic potential of honey-derived bioactive compounds for simultaneously treating DR and AMD using bioinformatics-driven network pharmacology and molecular modeling approaches. A comprehensive literature review identified 170 bioactive compounds from different honey types, which were screened using Swiss ADME for drug-likeness and pharmacokinetic properties. Gene expression datasets related to DR and AMD were retrieved from the GEO database and analyzed through GEO2R to identify differentially expressed genes (DEGs). A Venn diagram identified common target genes between the two diseases. Cytoscape was used to construct bioactive-disease networks and analyze protein-protein interactions (PPI). Key hub genes were identified using the CytoHubba plugin. Molecular docking simulations were conducted with PyRx to evaluate binding affinity between bioactive compounds and target proteins. Five key bioactive compounds, taxifolin, morin, epicatechin, rhamnetin, and quercetin, exhibited strong interactions with hub genes such as EP300, BCL2, and HIF1A. Enrichment analysis revealed involvement in critical pathways, including HIF-1 and calcium signaling. Docking simulations confirmed stable binding with a low energy score. This study highlights the therapeutic potential of honey bioactives in targeting multiple pathways involved in DR and AMD. The results suggest that

these compounds could serve as promising candidates for further experimental validation, offering multi-targeted treatment options for these vision-related diseases.

Keywords: *Diabetic retinopathy, age-related macular degeneration, bioactive honey, network pharmacology, molecular modeling*

PP-42

Role of Antiglycating Agents to Ameliorate Age-Induced Cardiotoxicity

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ABSTRACT

According to the WHO, diabetes mellitus is the most common health condition with 422 million affected individuals globally. This is a complex metabolic disorder associated with life-threatening complications, including diabetic retinopathy, various neurological disorders, nephropathy, and various cardiovascular complications. Among these complications, about 65% of morbidity and mortality caused by cardiovascular diseases are associated with diabetes, making it a big health challenge. Pathophysiological studies of diabetes suggest that advanced glycating end products (AGEs) produced during diabetes alters the cellular processes by binding with its specific membrane receptor, RAGE (Receptor for Advanced Glycating End Products). This AGE-RAGE nexus affects various cellular mechanisms and leads to multiple macrovascular and microvascular complications. The deleterious effects of AGE-RAGE binding also cause the inflammation of smooth heart muscles and thrombosis, leading to atherosclerosis. Our study aims to determine the synergistic effect of known antiglycating compounds against cardiotoxicity, mediated by AGEs in diabetes. To achieve this goal, the antiglycation activity of test compounds was investigated. This will be presented as a poster. Previously, our team also investigated the role of potent compounds in altering macrophages' polarization state in diabetic atherosclerosis (H Jahan, MI Choudhary, 2021 – Elsevier). We have also investigated the possible effect of triozoles against glucose- and methylglyoxal-AGEs-induced inflammation in human monocytes (Jahan *et al.*, Life Sciences, 291, 120282). We have also published a patent on glycation, carbonyl stress, and AGEs inhibitors (Jahan, H., & Choudhary, M. I. (2015). Expert opinion on therapeutic patents, *25*(11), 1267-1284).

Keywords: *Diabetes, AGEs, Oxidative stress.*

PP-43

Potential of Antiglycation Compounds Against AGEs-Induced Cardiomyocytes Injury

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ABSTRACT

Advanced glycation end products (AGEs) are the main contributors to the development of diabetes-associated late complications. AGEs are formed because of persistent hyperglycemia and lead to vascular damage and diabetic cardiomyopathy. AGEs' interaction with their receptors RAGE increases oxidative stress and cardiotoxicity. Herein, we studied the effect of newly discovered antiglycation compounds on cardiomyocytes by employing molecular techniques using myoblast cell line as a model. The test compounds exhibited a promising antiglycation effect at the cellular level and thus exhibited a potential for further investigation as therapeutic agents. The key results will be presented in the poster presentation. Suppression of COX-2/PGE2 levels by carbazole-linked triazoles via modulating methylglyoxal-AGEs and glucose-AGEs – induced ROS/NF-κB signaling in monocytes (Jahan *et al*., 2022). Gliclazide alters macrophages' polarization state in diabetic atherosclerosis in vitro via blocking AGE-RAGE/TLR4-reactive oxygen species-activated NF-kβ nexus (Jahan and Choudhary, 2021). Anthranilic acid derivatives: novel inhibitors of advanced glycation end-products (AGEs) formation (US Patent 9,381,182). Role of novel carbazole linked 1, 2, 3-triazole analogs in alleviating methylglyoxal-mediated late diabetic vascular complications (US Patent 11,925,622).

Keywords: *AGEs, Oxidative Stress, Myoblast, Diabetes*

PP-44

Bio Ceramics of Drug Delivery

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ABSTRACT

Bioceramics, such as calcium phosphate ceramics and cement and sillica-based glasses, are widely used as components of implants for bone and teeth restoration. Nowadays, the advanced processing methods and new chemical strategies allow the incorporation of drugs within them or their functional surface. In this regard, bioceramics act as a local drug delivery system to treat a large bone defect, Osteoporotic fractures, bone infection, and bone tumors. The development of new perspectives for

cancer therapies. Mesoporous silica nanoparticles can be prepared to release the drug within specific cancerous cells. When the pores are closed with molecular nanogates, stimuli-responsive systems can be obtained, thus allowing drug release at will by supplying external stimuli such as magnetic fields, Ultrasounds or light. The present review looks at the advances in the bioceramics drug delivery system, as well as those nanoceramics intended for specific and controlled drug release. Bio ceramics-based implants are required to replace damaged hard tissue, i.e, bone and teeth. These new compositions must tackle bone repair in a more efficient and durable way than the current therapies. The current scenario regarding treatment of musculoskeletal disorders is that 2.2 million. Basically, a drug delivery system can be described as a formulation that controls the rate of drug delivery and targets specific areas of the body.

Keywords: *Calcium phosphate ceramics and silica-based glasses, Osteoporotic fracture tumor, cancer therapies, drug delivery*

PP-45

The Valorization of Hop Shoots (*Humulus lupulus*) for Their Phenolic Content and Free Radical Scavenging Activity

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ABSTRACT

The tough, dioecious perennial climbing plant known as hop shoots (*Humulus lupulus*) is a member of the *Cannabinaceae* family. In some parts of Pakistan, especially in the province of (Hunza), Gilgit Baltistan, which is the country's major hop-growing region, hop shoots also referred to as hop sprouts are considered a delicacy. Its high value makes it well-known in the brewing industry. The presence of several phytochemicals, including prenylflavonoids like xanthohumol and other phenolic compounds like flavonoids and tannins, gives hop shoots their strong antioxidant activity. By efficiently scavenging free radicals, these substances can stop chain reactions that harm cells and tissues through oxidative damage, which could help prevent chronic illnesses, including cancer, heart disease, and neurological disorders that are connected to oxidative stress. This study sets out to assess the antioxidant activity and total polyphenolic content of hop shoot's methanolic extract. The Folin-Ciocalteau reagent was used to measure the extracts' phenolic content, and the DPPH assay was used to assess their antioxidant activity. The dried hop shoot's phenolic content, measured in gallic acid

equivalents, was 9.5±0.7 mg/g, and the free radical scavenging activity percentage inhibition varied between 15.7±1.8-65.53±4.1 at concentrations 0.2-1.0 mg/ml. The study's findings demonstrated that hop shoots' methanolic extracts have strong antioxidant properties, suggesting a promising future for natural antioxidants in food, pharmaceuticals, and medicine.

Keywords: *Antioxidant, Phenolic content, Free radical, Hop shoots.*

PP-46

Development of Kojic Acid Toner

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ABSTRACT

Kojic acid, a secondary metabolite derived from Aspergillus and Penicillium species, is widely recognized for its skin-brightening properties due to its ability to inhibit tyrosinase, a key enzyme in melanin synthesis. This study focuses on the formulation and characterization of an organic kojic acid toner aimed at reducing hyperpigmentation while maintaining skin hydration and safety. The toner was developed using a stable kojic acid complex combined with humectants and botanical extracts to enhance efficacy and skin compatibility. Stability studies, pH analysis, and microbiological testing were conducted to ensure product safety. A clinical study was performed on participants with hyperpigmentation, evaluating skin tone improvement, moisture retention, and irritation potential over 8 weeks. Results showed a significant reduction in dark spots and an improvement in overall skin brightness, with minimal irritation reported. The eco-friendly formulation aligns with the growing demand for sustainable and effective skincare solutions.

Keywords: *Kojic acid, hyperpigmentation, toner brightening, tyrosinase inhibition, eco-friendly skincare*

PP-47

Assessment of Biological Activities of Secondary Metabolites of *Pseudomonas chlororaphis* subsp. *Aurantiaca*

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ABSTRACT

The need for sustainable and optimal drug formulations to combat drug resistance is escalating. Microbial secondary metabolites are products with unique structures and low molecular mass. Pseudomonas species contain a wide range of secondary metabolites with versatile biological activities such as phytotoxic, antimicrobial, antimitotic, herbicidal, nematocidal, and signal molecules that sense. quorum. The current study was performed to assess various biological activities of phenazine fractions obtained from Pseudomonas Chlororaphis. The extraction was done using Ethyl acetate, n-Hexane, Dichloromethane, Chloroform, Methanol which was followed by LC-MS analysis of the fractions to authenticate the presence of phenazines. Biological evaluation of the fractions was performed via DPPH assay, Disc diffusion assay, and antityrosinase assay. The antimicrobial assay was performed against the following bacterial pathogens i.e. Staphylococcus aureus, Streptococcus viridians, Streptococcus pyogenes, Enterococcus faecalis, Coagulase- negative Staphylococci, Pseudomonas aeruginosa, *Escherichia coli*, Klebsiella pneumoniae, Acinetobacter baumannii. A hemolytic assay was performed to evaluate the cytotoxicity of the extracts. In silico analysis of the identified compounds were done with tyrosinase to predict the interactions. LC-MS analysis showed the presence of the following phenazines Mupirocin/Pseudomonic acid, Phenazine-1-carboxylic acid, Pyoluteorin, 2-OHPhenazine. The highest antioxidant activity was shown by ethyl acetate extract with IC50 value of 10.72 µg/mL. The highest antibacterial potential was observed with the chloroform extract with lowest MIC obtained against gram-negative bacteria Acinetobacter baumannii i-e 24.66 µg/mL ± 1.52. Among the five fractions, the strong Antityrosinase activity is observed in methanol fraction with IC50 of 0.29 µg/mL among others with comparable activities. The *in-silico* analysis has shown strong interactions of Mupirocin/Pseudomonic acid with tyrosinase with the highest Vina score ie -6.9 kcal/mol. Further validation in vivo analysis is strongly recommended to take a step forward in drug development.

Keywords: *PGPRs; Pseudomonas chlororaphis; Antityrosinase activity; Antioxidant activity; Antibacterial activity*

PP-48

In Vitro Antioxidant, Antimicrobial & Phytochemical Analysis of Ethanolic Extracts of *Brassica Nigra*

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ABSTRACT

Mustard plants are any of a few plant categories in the genera Brassica and wild mustard in the family *Brassicaceae*. Mustard seed is utilized as a flavor. Crushing the seeds with water, vinegar, or different fluids makes a mix. The seeds can likewise be squeezed to make mustard oil and can be eaten as mustard greens. The purpose of this study was to investigate the antimicrobial activity and Phytoconstituents qualitatively present in different parts of *Mustard plant* and to *investigate* antioxidant activity. The cold maceration process was used on seed, flower, stem and leaves of *Mustard* plant in ethanol. Extracts were then prepared to perform antibacterial, phytochemical analysis and antioxidant activities. The Antibacterial effect of *Mustard plant* and their synergistic effect on antibiotic drugs was evaluated by ethanolic extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella enterica and Klebsiella pneumonia*. The antibacterial effects were looked over by using well diffusion and disc diffusion method. The 25mg extract of mustard seed (Mu-01) showed more zone of inhibition against as Proteus (21mm) and in 50mg extract of Mu-01 shows best zone of inhibition against S. aureus (34mm). In synergistic effect the extract was made using ethanol and ethyl alcohol. Comparatively 25mg/1ml DMSO showed less results than 50mg/1ml DMSO results. Qualitative phytochemical screening showed the presence of phytochemicals present in ethanolic extracts. DDPH radical scavenging activity assay revealed the distinguished antioxidant activity of *Mustard seed and stem than other extracts.* Experimental results of *Brassica Nigra* seed and stem possessed good antimicrobial activity. *Brassica Nigra* plant contains secondary metabolites phenol, flavonoids, alkaloids, sterols, terpenes. It has commercial interest in both research institutes and pharmaceuticals industries for manufacturing of new drugs for the treatment of different diseases. Seed extract is a promising candidate for use as natural products-based antioxidants for the health of human beings.

Keywords: *Mustard plant, antimicrobial activity, Phytochemical screening, Antioxidant properties, Brassica Nigra, Synergistic effect.*

PP-49

Nutritional Miracles of *Moringa oleifera* for Betterment of Human Health

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ABSTRACT

Moringa oleifera is a multi-purpose herbal plant used as food and for medicinal purposes for the betterment of human health. Recently, Moringa oleifera leaves have wide applications as nutraceutical agents in the food supplementation industry. Biological activities of different parts of plant like anti-inflammatory, anti-bacterial, anti-cancerous, and anti-diabetic have been reported and considered as superior among other health benefits. The great range of nutritional and therapeutic usages of Moringa oleifera proves to be a valuable source of potential phyto-constituents with diverse functionality. It has a valuable impact towards the control of climate change. Due to its significant role in nutritional, medicinal and industrial fields, Moringa oleifera has great economic values. It can be declared as a "Miracle Tree" because of the presence of abundant nutrients with an excellent feeding impact and a high protein biological value. The moringa tree is a useful tool in the prevention of global warming because it sequesters more atmospheric carbon with its all parts. Moringa oleifera, the "Miracle Tree", has a lot of potential for use in the food and medicine industry but hasn't been fully realized. In general, the Moringa oleifera tree can be considered best in providing benefits to humans in almost all aspects such as food, medicinal, industrial, agricultural and environmental. It presents very exciting opportunities for farmers in the areas of foliar spray, green manure, natural fertilizer, fodder, soil and water conservation. It also helps farmers to use less water and reduce greenhouse gas emissions. The ability of Moringa tree to sequester more atmospheric carbon with all its parts and ultimately prevents global warming is very inspiring. Hence, planting Moringa oleifera will mitigate the negative effects of climate change, which is needed of the globe in the present era. Because of its low cost and a lot of benefits, Moringa oleifera is truly a wonderful "Gift" for humanity.

Keywords: *Moringa oleifera, miracle tree, health benefits, nutraceuticals, antioxidant activity, climate change.*

PP-50

Biopotential of Purified Bioactive Compounds Derived from *Citrullus colocynthis*

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ABSTRACT

Citrullus colocynthis has been known for its medicinal properties throughout history. This plant contains various bioactive compounds that contribute to its diverse biological activities, including anti-diabetic, anti-oxidant, and anti-inflammatory properties. This study aimed to extract, purify, and characterize bioactive compounds from *C. colocynthis* and evaluate its biological activities. The extraction process involved soaking dried plant material in organic solvent, followed by filtration and solvent evaporation. Purification of bioactive compounds was done by flash column chromatography using silica gel as the stationary phase and a gradient solvent system. Thin-layer chromatography (TLC) was used for preliminary compound identification, followed by liquid chromatography-mass spectrometry (LC-MS) for detailed phytochemical profiling. The biological efficacy of the purified fractions was assessed through antioxidant, anti-diabetic, and anti-inflammatory assays. This study showed that the ethyl acetate fraction demonstrated highest anti-diabetic, anti-oxidant, and anti-inflammatory properties. *Citrullus colocynthis* bioactive compounds have the potential to be utilized in the creation of novel drugs and therapies, providing a more sustainable approach to healthcare.

Keywords: *Citrullus colocynthis, Medicinal plant, antioxidant, anti-diabetic, and anti-inflammatory*

PP-51

In silico Vaccine and Drug Designing against MAPK Protein Express for Alzheimer's Disease in a Zebrafish Model

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ABSTRACT

Alzheimer's disease (AD) is a chronic neurodegenerative disorder due to the overproduction or accumulation of Aβ (Amyloid β) plaques, which causes an inhibition of phosphorylation of proteins

that regulate signaling pathways. Understanding the role of the mitogen-activated protein kinase 1 (MAPK1) signaling pathway is critical in advancing treatments against neurodegenerative diseases such as Alzheimer's. In this study, we employed In-silico approaches to retrieve and analyze the MAPK1 protein sequence, facilitating structural characterization, evolutionary assessment, and population distribution analysis. Using advanced computational tools, we predicted cytotoxic T lymphocyte (CTL) epitopes and evaluated their immunogenicity and allergenicity to assess their potential for vaccine development. We identified 30 cell-targeting molecules, from which the top 10 CTL epitopes (*PAGGGPNPG, GGGPNPGSG, SAPAGGGPN, AVSAPAGGG, AGGGPNPGS, ATAAVSAPA, TAAVSAPAG, ENIIGINDI, INDIIRTPT, and NDIIRTPTI*) were selected for further investigation based on their high binding affinity and immunological relevance. Notably, these epitopes demonstrated extensive global population coverage, with predicted efficacy rates of 88.5% and 99.99% for different vaccine formulations. Furthermore, molecular docking studies were conducted using a library of compounds from the ZINC database to identify potential inhibitors targeting MAPK1. Twelve compounds with the lowest binding energy were identified, interacting with key MAPK1 residues, including *VAL48, LYS63, CYS175, ASP176, LYS160, ALA61, LEU165, TYR45, SER162, ARG33, PRO365, PHE363, ILE40, ASN163,* and *GLU42.* These interactions suggest a potential regulatory effect on MAPK1 activity. Our findings indicate that the identified peptides exhibit strong binding affinity and stability in complex with MAPK1, supporting their potential as therapeutic inhibitors. This study provides valuable insights into drug discovery and computational screening, offering promising avenues for the development of novel therapeutics aimed at mitigating MAPK1 hyperactivity associated with Alzheimer's disease.

Keywords: *Alzheimer; Zebrafish: Pharmacophore; Virtual Screening; Molecular Docking; Multiepitope; Vaccine Design; MAPK1.*

PP-52

The Biochemical Paradigm of Hope: A Medical Science Analysis of Logotherapy's Therapeutic Efficacy in Modulating Neurotransmitter Systems for Enhanced Patient Health Care

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ABSTRACT

Since the advent of time, man has always been striving hard to improve his lifestyle, especially his health. This search for improvement has led mankind to advancements in the scientific realm, but in return, it has cost people his physical and mental health. It is true that Medical Science has advanced a lot and is helping a lot in the sector of Public Health. Still, some gaps need to be considered by all the researchers, doctors, paramedics and all the associated people of this field. This perspective article aims to provide a potentially strategic solution to that. Integrating Medical Science with Mental Science to enhance Neurotransmitters ensures that not only the body but the mind and soul are also rejuvenated, which is, without any doubt, the true essence of healing. By the integration of personal experience, narrative review of literature, and conceptual reasoning, this perspective article will investigate the potential of logotherapy in the field of Psychosomatics for improved patient health care. It was seen that Logotherapy enhanced the functioning of patients' Neurotransmitters. The rate of recovery from disease for the patients who got logotherapeutic treatment along with the prescribed medicine to counter their disease was much higher than for the patients who did not get it. It was also observed that patients who recovered in this way were in much better shape than before and led unexpectedly long lives. In a nutshell, we can conclude that integrating Logotherapy and Medical Science can do wonders for mankind. It is proven that the enhancement of patients' Neurotransmitter functioning through Logotherapeutic treatment improved the health of many patients to great extents. Further research must be done in this field to improve the health of millions of people all over the world.

Keywords: *Logotherapy, Neurotransmitters, Medical Science, Public Health, Psychosomatics.*

PP-53

Synthesis of Silica-Coated Magnetic Beads to Enhance their Capturing Ability of Small Fragments of DNA

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ABSTRACT

To enhance the capturing ability of small fragments of DNA, various technologies are used. An improved technology is needed for rapid capturing and purification of smaller fragments of DNA. In this work, the synthesis of silica-coated magnetic beads is presented. Magnetic bead technology is extensively used to absorb and modify nucleic acids and has attracted a lot of attention in biological, medicinal, diagnostic, and engineering disciplines. Silica magnetic beads are Fe_3O_4 magnetic beads

with a coating of silicon dioxide (SiO_2). The present investigation relates to a method of making sedimentation adjustment silica-coated magnetic beads, which includes the step of preparing a magnetic core, coating the magnetic core with silica to form silica-coated magnetic beads, and washing it serially with alcoholic solvent and water. Silica-coated magnetic beads bind to DNA fragments and help in the extraction and purification of nucleic acids. These beads are a quick and easy way to purify plasmid DNA for transfection or sequencing, as well as genomic DNA for research. The obtained particle size of silica-coated magnetic beads is 0.5-1 um, which provides the optimal size for attaching bio-molecules for research, purification, and functional studies. Magnetic beads are completely spherical, unlike most other magnetic beads. The average particle size distribution is comparatively uniform, and the smooth surface eliminates carryover of impurities common to rough surface beads. Because the silica magnetic beads are spherical and uniform, DNA purification can be performed with outstanding efficiency and reproducibility. The magnetic property of the bead allows an external magnetic field to be applied for quick separation. ***Keywords:*** *Magnetic beads, DNA purification, DNA fragments, Particle size, Silicon dioxide.*

PP-54

Novel Amide Derivatives as Efflux Pump Inhibitors: A Promising Strategy for Tackling Multi-drug Resistance in *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is an alarming bacterium that poses a significant challenge in hospital settings. Its ability to resist antibiotics makes it a leading cause of various diseases. Research has identified efflux pumps as the primary mechanism behind their resistance. This study explores the potential of new amides in combating *P. aeruginosa* strains that produce these counter-efflux pumps. Multi-drug-resistant *P. aeruginosa* isolated from a tertiary care hospital in Peshawar was confirmed using gram staining and biochemical tests. Antibiotic susceptibility testing was performed, and efflux pumps were identified through the ethidium bromide agar cartwheel method and confirmed by UV transilluminator. The anti-pseudomonal activity of novel amides against efflux pump-positive strains was assessed using agar well diffusion and micro broth dilution methods, including synergy testing with ciprofloxacin and gentamicin. The study revealed that three highly active efflux pump strains of *P. aeruginosa* were susceptible to novel amides. Specifically, ITC, ITD, ITE, and DEP inhibited the

efflux pump, while TEM-cu demonstrated strong antibacterial activity without inhibiting the efflux pump. The minimum inhibitory concentration (MIC) values for TEM-cu and DEP were 0.19 mg/ml, whereas ITC and ITE had MIC values of 0.78 mg/ml. Notably, ITE exhibited the lowest activity against efflux pump-expressing *P. aeruginosa*, with an MIC value of 1.56 mg/ml. The study also found that combining TEM-cu and DEP with ciprofloxacin and gentamicin enhanced antibiotic efficacy. In conclusion, TEM-cu has shown promise in combating *P. aeruginosa* efflux pump strains, while novel amides (ITC, ITD, ITE, and DEP) have demonstrated efflux pump inhibition. The combination of DEP and TEM-cu with ciprofloxacin and gentamicin has yielded notable reductions in MIC values. This approach offers a promising solution for addressing efflux pump-mediated resistance in *P. aeruginosa* infections.

Keywords: *Antibiotic resistance, Efflux pumps, multi-drug resistant, Novel amides*

PP-55

Chitosan-Based Hydrogels for pH-Responsive and Controlled Release of Vitamin D3

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ABSTRACT

The prevalent Vitamin D3 deficiency attributed to its limited bioavailability in oral intake emphasizes the need to develop a sustained-release system. In this study, biocompatible and biodegradable chitosan-sago starch hydrogels (CS 1-4) as sustainable nutraceutical carriers for controlled Vitamin D3 release have been synthesized. The synthesized hydrogels were characterized using FTIR, XRD, and SEM to confirm their structural integrity. Swelling studies demonstrated their pH-responsive behavior, enabling a targeted and sustained release mechanism. Kinetic analysis revealed that Vitamin D3 release predominantly follows zero-order kinetics, indicating a controlled release mechanism significantly influenced by the concentration of chitosan. These prepared samples were an eco-friendly alternative for improving Vitamin D3 bioavailability, aligning with the principles of sustainable healthcare solutions.

Keywords: *Vitamin D3, Chitosan, Nutraceutical Carrier, pH-Responsive, Controlled Release.*

PP-56

A Review on the Development of Bio-Based Vitamin C Serum

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ABSTRACT

The growing demand for natural, sustainable skincare solutions has led to the development of a novel Vitamin C serum produced through the fermentation of Aspergillus niger using agricultural waste, specifically peanuts, as a substrate. Traditional Vitamin C serums often face challenges such as chemical synthesis or instability, rendering them ineffective for many consumers. This innovation addresses these issues by utilizing bio-based fermentation processes to produce Vitamin C in large quantities, without depleting natural resources. The serum is a clean beauty product, formulated to be suitable for all skin types, organic, biodegradable, and environmentally friendly. By employing agricultural waste, this process supports sustainability and offers an eco-conscious alternative to conventional skincare products. The serum's stable formulation ensures consistent efficacy, making it a viable solution for consumers seeking both effective and eco-friendly skincare options. The commercial strategy focuses on launching the product on e-commerce platforms with eco-friendly packaging, supported by social media campaigns, influencer partnerships, and endorsements from dermatologists. This approach targets individuals who prioritize natural ingredients, clean beauty, and environmentally sustainable products. Through these efforts, the Vitamin C serum, branded as PURE C**,** aims to revolutionize the skincare industry by offering a bio-based, stable, and effective solution for consumers seeking environmentally responsible beauty products.

Keywords*: Biotechnology, Sustainability, Fermentation, Natural Ingredients, Clean Beauty, Agricultural Waste.*

PP-57

BioHydraGel: Revolutionizing Skincare through Biotechnology

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ABSTRACT

BioHydraGel is a revolutionary skincare innovation utilizing biotechnology to produce sustainable and cruelty-free hyaluronic acid (HA). Unlike traditional HA, which is derived from animal sources,

BioHydraGel is synthesized through microbial fermentation, specifically using genetically optimized microbes like Streptococcus zooepidemicus. This biotechnological approach eliminates ethical concerns and significantly reduces environmental impact while maintaining high efficacy and purity. The production process involves bioreactors, where microbes convert simple sugars into HA, followed by advanced purification techniques such as chromatography to ensure product quality. BioHydraGel is formulated into a moisturizing gel that delivers deep hydration and skin repair without animal-based components. With the global HA market projected to reach $13 billion by 2030, the demand for ethical, eco-friendly, and sustainable skincare solutions is rapidly growing. BioHydraGel aligns with these market trends, setting new industry standards in ethical skincare. This innovation not only meets consumer preferences for vegan and cruelty-free cosmetics but also represents a pioneering advancement in biotechnology-driven skin care solutions.

Keywords: *Biotechnology, Hyaluronic Acid (HA), Microbial Fermentation, Cruelty-Free, Skincare, Sustainable Cosmetics, BioHydraGel, Ethical Skincare, Fermentation Bioreactors, Chromatography Purification*

PP-58

Production of a Recombinant Thrombolytic Agent using Streptokinase and Tissue-type Plasminogen Activator

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ABSTRACT

Reteplase and Streptokinase function as thrombolytic agents by activating plasminogen, ultimately leading to fibrin degradation and clot dissolution and cleaving it at the Arg561-Val562 site. In this study, recombinant hybrid proteins were designed for clot lysis and expressed in *Escherichia coli* using recombinant DNA technology. *In silico* modeling of reteplase (K+P), Kringle 2-SKC, and P domain-SKC were performed, followed by structure refinement using GalaxyRefine. Molecular docking with plasminogen was carried out using ClusPro, and Ramachandran plot analysis confirmed that most residues were in favored regions, indicating structural stability. In the experimental phase, the genes encoding reteplase (K+P) and Kringle 2-SKC were successfully cloned into *E. coli* expression vectors *pET-28a* and *pET-22b,* respectively. The P domain-SKC construct was cloned into the pTZ57R/T cloning vector for further studies. The recombinant constructs were designed to enhance thrombolytic activity, improve protein stability and reduce immunogenicity. This study combined computational

and experimental approaches to develop potential therapeutic proteins for clot lysis. Future studies will focus on optimizing expression, purification, and functional characterization of these hybrid thrombolytic proteins.

PP-59

Synthesis of Copper Oxide Deposited Covalent Triazine Framework for the Colorimetric Sensing of Uric Acid

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ABSTRACT

Uric acid is a product of purine metabolism in the body. The normal range of UA is essential for health and wellness. For accurate detection of the U.A., many methods are used, which is previously reported. But in recent years, Covalent Organic Framework (COF), which is crystalline porous material, has received very significant attention. For the first time, we synthesized the CuO-deposited covalent triazine framework (CuO@CTF) with the Co-precipitation method. For the preparation of CuO@CTF, we take Dicyanobenzene and zinc Chloride on 1 :1 and heat up at 400 oC in the furnace. The obtained product washed many times with HCl to ensure the neutral pH for further processes, filtered the solution and dried at 1 500C for 30 minutes in oven and then for the deposition of Copper Oxide, we take Copper Sulfate and Covalent triazine framework in 1 :9. In this stage we proceed the Co-precipitation method for getting Copper Oxide deposited Covalent triazine Framework CuO@CTF. Then, ensure the neutral pH and dried the solution at 60 oC for 5 hours, while the successfully prepared nanocomposite CuO@CTF are used for the accurate colorimetric determination of uric acid. We perform the UV-Vis Spectroscopy for the conformation of Uric acid detection, nanocomposite stability and sensitivity, and detection limit and selectivity. The fabricated Cu@CTF was characterized by using many other techniques, including X-ray diffraction (XRD), which was used to confirm the nanocomposite crystalline structure, while FTIR Analysis was performed to identify the characteristic peaks of the material, and Scanning Electron microscopes (SEM) were carried out for the morphology of CuO@CTF nanocomposite. Additionally, XPS was performed for material surface chemistry. The notable efficiency of CuO@CTF nanocomposite as peroxidase mimic enzyme can be considered as noteworthy material for the purpose of colorimetric determination of Uric acid and biological applications.

Keywords: *Dicyanobenzene, Zinc Chloride, HCl, NaOH, Copper Sulfate, colorimetric determination of Uric Acid.*

PP-60

Structural and Functional Insights into *Bacillus licheniformis* α-Amylase Variants through Protein Engineering Strategies

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ABSTRACT

Alpha-amylases are vital enzymes that hydrolyze α-1,4-glycosidic bonds in starch, generating glucose, maltose, dextrins, and oligosaccharides, making them indispensable in various industrial applications. This study explores the structural and functional dynamics of *Bacillus licheniformis* α-amylase variants (BLAMWSP and BLAMCD) through N- and C-terminal truncations and site-directed mutagenesis (Thr353Ile and His400Arg). Molecular dynamics simulations validated the stability of catalytic and substrate-binding residues, corroborating molecular docking predictions. Circular dichroism spectroscopy and temperature ramping assays confirmed that both variants retained structural integrity at 90 °C. Kinetic analysis revealed specific activities of 2343.09 ± 0.20 and 4237.88 ± 0.66 μmol min^{-1}/μmol protein for BLAMWSP and BLAMCD, respectively, at 90 °C in 100 mM phosphate buffer (pH 6.0). The BLAMCD variant exhibited a two-fold increase in enzymatic activity, attributed to enhanced substrate accessibility at the active site. Comparative catalytic efficiency (kcat/Km) values of 51.76 ± 1.76 and 114.10 ± 1.41 further established BLAMCD's superior performance. These findings underscore BLAMCD's potential as a highly efficient biocatalyst for industrial starch liquefaction and saccharification.

Keywords: *Bacillus licheniformis, α-amylase, protein engineering, catalytic efficiency, starch hydrolysis.*

PP-61

Engineering the Laccase-Xylanase Chimera from *Bacillus subtilis* Strain R5

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ABSTRACT

Enzymes play a crucial role in various industrial applications, including food processing, pulp, and paper manufacturing, and biomass utilization. They are known for their ability to catalyze specific reactions, allowing for efficient and cost-effective processes. However, there is still room for improvement in terms of enzyme efficiency and versatility. Multi-functional enzymes allow efficient substrate hydrolysis by limiting their diffusion and enhancing spatial proximity. This study aims to use wild-type Laccase and Xylanase genes from a Bacillus subtilis Strain R5 to synthesize a chimeric enzyme for various industrial applications. Constructing a chimeric enzyme by fusing Laccase and Xylanase domains may enhance the conversion of complex polymeric structures of plant cell walls into mono- or oligomeric molecules, leading to improved process efficiency and reduced resource consumption. In the pulp and paper processing industry, Xylanases and Laccases are involved in the bleaching and delignification processes. To optimize the expression of the chimeric enzyme, multiple chimeric combinations were prepared by silico methods. Furthermore, the enzymatic properties and catalytic efficiency of the chimeric enzyme were studied by conducting biochemical assays. Moreover, the stability and performance of the chimeric enzyme were assessed under various conditions, such as temperature, pH, and substrate concentration.

Keywords: *Laccase-Xylanase Chimera; Catalytic efficiency; cost-effective; Industrial applications.*

PP-62

A Comparative Study of Native and Engineered Xylanase *Bacillus subtilis* R5

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ABSTRACT

Xylanase belongs to the glycosyl hydrolase family that hydrolyzes complex polysaccharides into the simple sugars, i.e., the conversion of xylan to xylose. Xylan is a major component of hemicellulose, a polysaccharide found between the cellulose fibers and the plant cell wall matrix lignin. The β-1,4 glycosidic bonds in xylan are hydrolyzed by the action of Xylanase. *Bacillus subtilis* strain R5 is categorized under the glycosyl hydrolase family (GH11). pET-28a(+) vector was used for cloning and

expression of native and engineered Xylanase gene. Six N-terminal amino acid residues after the signal peptide were deleted in engineered Xylanase. Both proteins were produced in soluble form in Escherichia coli Rosetta (DE3) at low temperatures, but a small amount of native Xylanase was also produced in the soluble form at 37°C. Native and engineered proteins were produced on a large scale and purified by Ni-NTA chromatography. Further, purification was performed by the dialysis of both proteins separately in 50 mM Tris-Cl (pH 8.0). Purified proteins were used in the Xylanase enzymatic activity assay. The activity of native Xylanase was two-fold higher than the engineered Xylanase, indicating the significance of the first six N-terminal residues in enzyme stability and activity. Further research is necessary to compare and optimize the catalytic activity and thermostability of these enzymes.

Keywords: *Protein engineering; Xylanase; Bacillus subtilis R5; Glycosyl Hydrolases*

PP-63

Engineering of Thermostable L-Asparaginase for Enhanced Acrylamide Mitigation

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ABSTRACT

Acrylamide, a potential carcinogen formed during high-temperature food processing, arises from the Maillard reaction between reducing sugars and L-asparagine. L-asparaginase effectively mitigates acrylamide formation by hydrolyzing L-asparagine before it can participate in this reaction. Industrial strategies, such as blanching followed by L-asparaginase treatment, can reduce acrylamide levels by up to 90%. The archaeal L-asparaginase Tk1656, with optimal activity at 85°C and pH 9.5, requires enhanced thermostability for large-scale food processing. Site-directed mutagenesis generated variants with high frequency, including T17G, M19E, L56D, T55Q, and K299L. Combinatorial mutants (M19E-T55Q, T17G-K299L, M19E-K299L, and T55Q-K299L) were further developed to enhance performance under industrial conditions. Activity analysis from 37–95°C identified 90°C as the optimal temperature, with M19E-K299L and T55Q-K299L maintaining superior activity at 90-95°C, indicating increased thermostability. pH assays at 90°C (pH 7 and 9.5) revealed significantly enhanced activity for all mutants except M19E-T55Q. Notably, T55Q-K299L exhibited a 150% activity increase, making it a promising candidate for acrylamide mitigation in high-temperature food processing environments. These findings highlight T55Q- K299L as a robust enzyme for reducing acrylamide in heat-processed foods, offering a scalable and effective solution for improving food safety.

Keywords: *Acrylamide mitigation, L-asparaginase, Maillard reaction*

PP-64

In Vitro Half-Life of Human Alpha-2b Interferon Derivative (PHE-IFN-B_5) in Plasma using ELISA

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ABSTRACT

Interferons serve as the first line of defense against viral infections due to their immunomodulatory properties, which make them vital in the biopharmaceutical industry. In SBS, several derivatives of interferon were recombinantly prepared. This study aimed to establish a standard optimised protocol for determining the *in vitro* half-life of interferon derivatives in plasma under physiological conditions. For this purpose, the Phe-ifn-b_5 derivative was designed by engineering a chimera of human interferon alpha-2b and cytochrome b_5, having a molecular weight of 31.03 kDa. Phe-ifn-b_5 was selected for half-life studies because of its integrity at N-terminal region with no heterogeneity and slightly larger in molecular weight as compared to wild type interferon alpha-2b (19.396 kDa). Hence giving it much more probability to sustain under *in vitro* conditions. Indirect ELISA showed the following optimized conditions: anti-Phe-ifn-b_5 raised in mice showed 1.5 times stronger titre than in rabbits, sensitivity was 0.5–1 ng using a titre of 1000X dilution of anti-Phe-ifn-b_5 raised in mice, and 4000X dilution of anti-mice IgG peroxidase as a secondary antibody. Sandwich ELISA demonstrated greater sensitivity when rabbit and mouse antibodies were used as the primary and secondary antibody systems, respectively. For *in vitro* half-life analysis, Phe-ifn-b_5 (5 µg/ml) was incubated in fresh human blood at 37 °C for 8 hours with the addition of 0.4 mM EDTA. 100 µl of sample was aliquoted, plasma was extracted, and 100X diluted plasma was used to monitor the half-life of Phe-ifn-b_5. Indirect and sandwich ELISA indicated a gradual decline in signal with a half-life of approximately 4 hours. This research provides a systematic protocol for investigating the *in vitro* half-life of a drug in biological samples using interferon as a model protein, which facilitates designing *in vivo* half-life assays and pharmacokinetics studies of not only interferon derivatives but other biopharmaceuticals as well.

Keywords: *Interferon, in vitro half-life, ELISA, Pharmacokinetics, Immunoassay.*

PP-65

Identification of Prognostic Biomarkers in Breast Cancer Using Multi-Omics Data Mining

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ABSTRACT

Breast cancer is a malignant disease and is the most common cancer in women globally. Its prevalence in Pakistan is among the highest in Asia, posing a significant health challenge. In this study, we aim to identify hub genes with potential as prognostic biomarkers for breast cancer. For this purpose, we downloaded four datasets (GSE42568, GSE10810, GSE45827, and GSE65194) from NCBI Gene Expression Omnibus. We used R studio to identify differentially expressed genes (DEGs) followed by functional enrichment analysis using DAVID database. Enrichment analysis revealed significant pathways, including the AMPK signalling pathway, PPAR signalling pathway, ECM receptor interaction, and Adipocytokine signalling pathway. These DEGs were then used to construct a protein-protein interaction network, which was further analysed to identify highly correlated genes using MCODE and CytoHubba. Six hub genes, including AURKA, BUB1B, CCNB1, CCNB2, CENPF, and MELK, were identified and validated via Kaplan-Meier survival analysis. Subsequently, coexpression analysis was conducted to support further studies. The findings highlight key molecular targets that could improve breast cancer prognosis and establish these genes as biomarkers for personalized therapeutic strategies.

Keywords: *Multi-omics, Breast cancer, DEGs, Biomarkers.*

PP-66

Discovery and Design of Antimicrobial Peptides (AMPs) for Potential Lung Cancer Therapy using Multi-Omics and Machine Learning

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ABSTRACT

Lung cancer remains a leading cause of cancer-related mortality worldwide, necessitating novel therapeutic interventions. The search for anticancer medicines from natural sources has continued to gain popularity because of their diverse range of bioactive compounds. This research explored the potential of antimicrobial peptides (AMPs) as a novel lung cancer therapy, employing a multi-omics and machine learning approach. Biosynthetic gene clusters from 13 microbial samples, including bacteria, fungi, probiotics, and metagenomic sources, were analyzed using antiSMASH for secondary metabolite prediction. The CAMPR3 tool was used to identify potential AMPs, which were screened based on the highest likelihood scores predicted by 3 machine learning tools: support vector machine (SVM), artificial neural network (ANN), and random forest (RF). AMPs were identified, reconstructed, and screened for anticancer activity using AI-driven software AntiCP1 and AntiCP2, which uses SVM to find the anticancer potential based on amino acid sequences. Based on physiochemical properties, such as hydrophobicity, different types of amino acids, GRAVY score, instability index, Bowman index, aliphatic index, and potential anticancer peptides were analyzed. Heatmaps, correlation maps, and principal component analysis (PCA) played a key role in understanding the relationship between different physiochemical properties for filtering the most potent anticancer peptides. A total of 45 potential anticancer peptides (ACPs) were identified. This study identified several promising peptides with potential anticancer activity, demonstrating the effectiveness of the multi-omics and machine learning approach in discovering novel therapeutic candidates. Further studies, including 3D structural prediction, molecular docking, and molecular modeling, are required to validate the results.

Keywords: *Antimicrobial peptides (AMPs), anticancer peptides (ACPs), lung cancer therapy, AI-driven screening, machine learning approach, natural products.*

PP-67

Use of Her2 to Treat Breast Cancer

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ABSTRACT

HER2-positive (HER2+) breast cancer, characterized by utilizing the overexpression or amplification of the human epidermal boom aspect receptor 2, represents a clinically huge subtype related to competitive tumor behavior. but the improvement of targeted treatment plans has dramatically stepped forward outcomes for those sufferers. This abstract summarizes the current landscape of HER2-centered healing procedures, encompassing monoclonal antibodies (e.g., trastuzumab, pertuzumab), antibody-drug conjugates (ADCs) (e.g., trastuzumab deruxtecan, T-DM1), and tyrosine kinase inhibitors (TKIs) (e.g., lapatinib, neratinib, tucatinib). we can discuss the evolution of treatment strategies, consisting of adjuvant, neoadjuvant, and metastatic settings, and highlight the significance of customized strategies, thinking about elements inclusive of disorder level, patient comorbidities, and resistance mechanisms. moreover, we will cope with emerging studies regions, which include novel HER2-targeted sellers, strategies to triumph over resistance, and the integration of immunotherapy, aiming to optimize treatment efficacy and enhance the pleasant of lifestyles for individuals with HER2+ breast cancer.

Keywords: *HER-2, Therapeutics, Breast Cancer.*

PP-68

Synthesis of Copper Oxide Nanoparticles Conjugated with Cytarabine and Estimation of Its Anticancer

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ABSTRACT

Cancer is the leading cause of death worldwide. The limitations of conventional cancer therapy include little to no selectivity and ineffective efficiency in the differentiation of malignant from healthy cells. Nanotechnology is a diverse field that contains different scientific and technological applications. Nanoparticles are very small particles that can't be seen with the naked eye. In this study, drug (CYT)-CuO Nanoparticles were synthesized by using the precipitation method. The characterization of CuONPs and their conjugation with drugs were done by Light microscope, UV-visible spectrophotometry, and FT-IR, and anticancer activities by MTT assays and immunohistochemistry.

Fluorescence observed under a microscope indicated the formation of CuONPs. FTIR analysis was performed for the binding of functional groups, and it also showed that phenolic, ketonic, methyl, ethyl, and carboxyl groups were present. MTT assay was performed for the anticancer activity of drug (Cytarabine) conjugated with CuO nanoparticles. Immunohistochemistry was performed for the binding of CYT- CuO nanoparticles with mouse epithelial tissues. According to results CuONPs synthesis, the brown color shows conformation of CuONPs conjugated with drug (Cytarabine). The UV- Visible range of drug-CuONPs was observed to be under 330nm. The FTIR analysis shows the binding under the 1700 to 1500 cm -1 . In MTT assays shows the high cytotoxicity of MCF-7 cells upon the concentration of drug at 50ug/mL and it shows the viability is 84%. Immunohistochemistry shows the binding of nanocomposites with mouse epithelial tissues that give the positive results in which folate is binding of folate receptors to cell surface. In future it can be used as an anticancer drug.

Keywords: *MTT assay, UV-visible spectroscopy, FTIR, Immunohistochemistry, CuO nanoparticles, Drug (Cytarabine).*

PP-69

Anti-Cancer Potential of *R. stricta* and Its Effect on De-novo Lipid Biosynthesis Pathway in MCF-7 Breast Cancer Cell Line

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ABSTRACT

Breast cancer has emerged as a major concern and is the most common type of invasive cancer among women globally. Plant-based treatment of breast cancer is considered safe compared to chemical-based treatment because plants do not affect normal parts of the body. *Rhazya stricta* is a plant that comprises different alkaloids, flavonoids, phytochemicals, and biologically active substances. This plant is employed to cure various diseases and may show anticancer activity against different carcinomas. This study aimed to find out the anticancer activity of the methanolic extract of *R. stricta* and its effect on the de novo lipid biosynthesis pathway in the MCF-7 breast cancer cell line. In this study, the methanolic extract of *R. stricta* was prepared to treat the MCF-7 breast cancer cell line. A series of plant extract dilutions (0µg/ml-300µg/ml) were applied to MCF-7 cells. MTT assay was performed to observe cell viability and to calculate the IC50 value of the extract. Cells were treated with an IC-50 dose of the extract (25µg/ml) for 72h. Treated cells were further evaluated by expression analysis of genes related to de novo lipid biosynthesis (FASN and ELOVL6). IC-50 value, and gene down-regulation indicated the anticancer potential of *R. stricta* and its ability to regulate de-novo lipid

biosynthesis in cancer. In conclusion, *R. stricta* can be a potential therapeutic agent to target lipid metabolism in breast cancer. Further research on molecular pathways can provide more insight into its mechanistic action.

Keywords: *Breast cancer, Rhazya stricta, Lipid Metabolism.*

PP-70

Role of Citrate Carrier (SLC25A1) in Tumor Progression

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ABSTRACT

The citrate carrier (SLC25A1), a mitochondrial transporter, is essential for lipid biosynthesis and energy metabolism. Emerging studies suggest that SLC25A1 overexpression in cancers enhances anabolic pathways, contributing to malignancy. However, the underlying pathophysiological mechanisms driving this dysregulation remain unclear. This study aims to investigate the role of SLC25A1 in tumor progression and its potential as a therapeutic target. To examine whether enhanced SLC25A1 expression induces tumor-like characteristics in healthy cells, a plasmid clone of SLC25A1 was transfected into the HEK293 cell line. Dysregulated lipid metabolism and oncogenic signaling were assessed through cell proliferation assays, lipid profiling, and expression analysis of selected genes. Gene expression analysis revealed an upregulation of the lipogenesis pathway, indicating that SLC25A1 facilitates a shift from canonical to non-canonical lipid metabolism. Increased cytoplasmic citrate promotes the conversion of citrate to acetyl-CoA, which fuels lipid synthesis. This was further supported by Oil Red O staining and lipid profiling results. Moreover, the expression of genes associated with the cell cycle, inflammation, and tumor progression indicated enhanced proliferation and inflammatory responses, reinforcing the role of SLC25A1 in cancer progression. This study concludes that SLC25A1 drives the reprogramming of lipid metabolism, leading to tumor-like properties in healthy cells. Thus, the current study suggests SLC25A1 is a promising therapeutic approach to disrupt cancer metabolism and suppress tumor growth.

PP-71

Enhancing Anticancer Efficacy through Synergistic Action of Zinc Nanoparticles and Walnut Extract

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ABSTRACT

The development of eco-friendly, natural-based therapies for breast cancer is gaining significant attention due to their reduced toxicity, cost-effectiveness, and enhanced therapeutic potential. This study focuses on the green synthesis of zinc nanoparticles (Zn NPs) using Aloe vera extract and zinc sulfate ($ZnSO_4$)**,** followed by their combination with walnut (*Juglans regia*) fruit extract to evaluate their cytotoxic effects on breast cancer cell lines. The synthesized Zn NPs exhibited optimal stability and uniform size distribution, as confirmed by spectroscopic and microscopic analyses. *In vitro* cytotoxicity assays demonstrated that the Zn NPs-walnut extract combination significantly reduced cell viability, with IC_{50} values markedly lower than those of individual treatments**,** suggesting a synergistic anti-cancer mechanism. The enhanced cytotoxicity is attributed to reactive oxygen species (ROS) generation, mitochondrial membrane depolarization, apoptosis induction, and inhibition of cancer cell proliferation**.** Additionally, the incorporation of Nigella sativa (black seed) extract, rich in thymoquinone, is proposed as a strategy to further potentiate the therapeutic efficacy of this combination by enhancing apoptosis signaling and oxidative stress induction. The findings highlight the potential of integrating green-synthesized nanoparticles with bioactive natural extracts as an alternative strategy for breast cancer treatment. Future research should focus on molecular mechanisms, in vivo validation, pharmacokinetics, and formulation optimization to facilitate clinical translation.

Keywords: *Green synthesis, Zinc nanoparticles (Zn NPs), Breast cancer therapy, Cytotoxicity, Reactive oxygen species (ROS), Apoptosis induction.*

PP-72

Genetic Identification, Structural Characterization, and Expression Analysis of KPCQ in Breast Cancer

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ABSTRACT

Protein kinase θ is a novel isozyme comprising 706 amino acids. The role of protein kinase theta has been explored in several different cancers like breast cancer, leukemia, gastrointestinal stromal tumors, and other cancers. The structure of KPCQ was determined and validated, and domains were analyzed through computational approaches. In-silico methodology was adopted for carrying out protein-protein docking and determining the hydrophobic as well as hydrophilic interactions amongst them. This study aims to investigate the possible role of KPCQ, p21 and c-jun in the progression of breast cancer. Two hundred samples were analyzed by real-time quantitative PCR for determining the relative expression of KPCQ, p21 and c-jun in breast cancer patients (stage I, II, III, IV). The target gene and interacting protein expression was also linked with the risk factors as well as clinical features of breast cancer. The study concluded that the expression of PRKCQ and c-jun was elevated in breast cancer patients. However, the tumor suppressor gene, p-21, expression was downregulated. The genes showed differential expressions when linked to risk factors and clinical features. Lastly, molecular dynamic simulations provided evidence related to the structural and functional changes due to the induced mutation. All this information is useful for early diagnosis and developing therapeutics for the treatment of cancer.

Keywords: *Breast Cancer, KPCQ, p21, c-jun, relative expression, diagnostic marker.*

PP-73

Unveiling the Anticancer Potential of Antarctic Yeast Extracts against Ovarian Cancer Cells

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ABSTRACT

Ovarian cancer is one of the leading causes of cancer associated mortality in women worldwide. About 70-80% of patients are diagnosed at a highly inclusive stage of growth. As we know, Antarctica is a geographical polar region and microorganisms over there have attracted attention as useful source for

novel therapeutics including anticancer drugs. So, this review paper investigates the effects of citromycin, isolated from the Antarctic marine-derived fungus Sporothrix sp., on human ovarian cancer cells. Other research agencies working on this are facing problems in storing this yeast as it thrives in extremely cold environments. Also, their growth rates are very slow, which limits their biomass yield. By using bioreactors and genetic engineering techniques, these problems can be solved. By critical analysis and narrative review of other research papers along with conceptual reasoning, this article will investigate how this Antarctic yeast affects patients with Ovarian cancer and how it can be obtained in larger quantities using specialized techniques. Citromycin inhibited the migration of human ovarian cancer cells and regulated the gene expression, highly impacting the symptoms of ovarian cancer positively. The symptoms were alleviated, and patients recovered from their cancer in due time due to this miracle fungus. Their yield was also increased using specialized procedures and bioreactors. Access to innovative cancer treatments could significantly increase survival rates and improve the quality of life. If production is scaled efficiently, Antarctic yeast-derived therapies might offer cost-effective alternatives to expensive cancer drugs, especially in low-income countries. Further research needs to be carried out into this field to make sure man makes the right use of Nature's gifts, just like this one.

Keywords: *Human ovarian cancer treatment, Antarctic yeast, Citromycin, Sporothrix sp.*

PP-74

Chicken-Derived Collagen for Enhanced Wound Healing

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ABSTRACT

Chronic wounds pose a significant healthcare burden due to prolonged healing times, risk of infection, and high costs associated with traditional treatments. Solution: This project proposes a novel approach to wound healing by developing a cost-effective and scalable method for extracting high-quality collagen from chicken byproducts (skin, tendons) using enzymatic techniques. This collagen will serve as the key ingredient in the wound healing cream. The extraction process involves thorough cleaning and size reduction of chicken byproducts, followed by enzymatic digestion using pepsin or trypsin to isolate collagen. Purification techniques like filtration and dialysis will be employed to remove impurities. The resulting collagen will be concentrated and dried using freeze-drying. This innovative solution targets individuals suffering from chronic wounds, healthcare professionals seeking effective treatments, and pharmaceutical companies developing novel wound-healing

products. The global wound care market is projected to reach USD 28.2 billion by 2027, indicating a significant demand for effective wound healing solutions. This collagen-based cream offers a natural, biocompatible alternative to existing synthetic products, potentially offering improved healing properties. Revenue streams include licensing collagen extraction technology, selling the collagen extract to manufacturers, and developing and marketing a consumer-ready collagen-based wound healing cream. This project presents a promising framework for developing a sustainable and cost-effective method for collagen extraction from chicken byproducts, leading to the development of a novel wound healing cream with the potential for a significant positive impact on patient outcomes. This approach addresses the technical challenges, secures funding, and navigates the regulatory landscape, paving the way for successful commercialization.

Keywords: *Collagen extraction, wound healing, chicken byproducts, enzymatic digestion, chronic wounds, biotechnology.*

PP-75

Propolis mediated Silver Nanoparticles for Wound Healing and Skin Regeneration

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ABSTRACT

Chronic wound infections and delayed healing have become major medical challenges. Thus, propolis-derived silver nanoparticles emerged as a sustainable, eco-friendly, and biocompatible solution to enhance the wound healing process. This study focuses on synthesizing propolis-derived silver nanoparticles and then blending them with a polymeric blend of Guargum and Sago starch. Characterization techniques including UV-VIS, FT-IR, SEM and XRD confirmed the spherical morphology and crystalline nature of prepared nanoparticles. These silver nanoparticles and polymeric blends exhibit significant antibacterial and anti-inflammatory properties. In vivo studies demonstrated that these silver nanoparticle-based ointments significantly facilitate the process of wound healing on burn injuries.

Keywords: *Wound infection, Propolis, silver nanoparticles, wound healing, ointment.*

PP-76

Curative Effect of *Aloe Barbadensis* Miller in Excisional Wounds Healing Mechanism Using a Rat Model

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ABSTRACT

A wound is a breakdown in the protective function of the skin or loss of continuity of epithelium, with or without loss of underlying connective tissues, muscles, nerves, bones following injury to the skin, surgery, a blow, cut, chemicals, heat, cold, friction, shear force, pressure or diseases such as leg ulcers or carcinomas. A study was undertaken to determine the healing properties of Aloe Vera gel on epidermal wounds in rats. Six adult rats were divided into three groups randomly of each group representing the treatment without treatment and control, respectively. A pair of wounds measuring 2cm x 2cm each was created on the back of each rat lateral to the spinal cord. The wounds were treated with homogenized Aloe Vera gel, while the wounds in the second group were treated with normal saline. Representing the inflammatory, proliferative, and maturation phases of wound healing, respectively. Blood samples were collected on day 21 for haematology analysis. Animals treated with Aloe Vera gel had significantly ($p<0.05$) faster rates of healing with shorter days of skin fall off than the control and untreated group. As showed significant ($P<0.05$) changes in the packed cell volume, mean corpuscular volume, lymphocyte and neutrophil counts. The study concluded that Aloe Vera was effective in treating epidermal wounds in rats than the control. An improvement occurred in the haematological profile of the experimental animals, and these findings will go a long way in expanding the horizon of clinical application of this plant in solving wound healing problems in both humans and other animal species.

Keywords: *Aloe Vera gel, Contraction, Epidermal wounds, Haematology, Topical application*

PP-77

Lymph Node-Based Organ Regeneration: Developments, Challenges, and Future Directions

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ABSTRACT

The regeneration of native organs has become a promising avenue in regenerative medicine, concerning the liver, thymus, kidneys and pancreas. Recent progress in tissue engineering and stem cell technology have demonstrated the capacity of lymph nodes to serve as micro-environment for organ regeneration. Because of distinct microenvironment and immune-privileged status, lymph nodes have become attractive locations for organ regeneration. With an emphasis on the liver, thymus, kidneys, and pancreas, this review examines recent developments in lymph node-based organogenesis. Progress in thymus, kidney, and pancreas regeneration is still mostly limited to small animal models, whereas liver regeneration has progressed to clinical trials (Phase 2a) because of the organs innate regenerative capacity and simpler functional requirements. Scalability, functional integration, and immune rejection are important issues that differ among organs because of their intricate structural and functional makeup. Despite these obstacles, organ regeneration based on lymph nodes has revolutionary potential for regenerative medicine. Large-scale animal research, clinical translation for organs other than the liver, and the incorporation of cutting-edge technologies like gene editing and personalized medicine are some potential future directions. This review highlights the promise of lymph node-based organogenesis as a revolutionary approach to addressing organ failure and chronic diseases

Keywords: *Regenerative Medicine, Lymph Nodes, Liver, Kidneys, Pancreas, Thymus, Renal Failure, Organogenesis, and Hepatic Failure.*

PP-78

Synthesis and Characterization of Novel Fenugreek-Infused Hydrogels

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ABSTRACT

The complete restoration of dermal skin injuries continues to present a substantial challenge in clinical wound healing and regenerative medicine. Self-healing hydrogels formed using polymeric materials have emerged as effective wound dressings owing to their inherent drug-carrying capacity, low cytotoxicity, low immunogenicity, and antimicrobial properties. In this research work, hydrogel-based wound dressing incorporating bioactive components and eco-friendly cross-linkers was prepared.

FTIR spectra provide strong evidence for cross-linking via borate networks, Schiff base formation, hydrogen bonding, and the presence of amide and carboxylic functional groups.GHF-20% showed the highest peak intensity in XRD analysis, suggesting that the components merge effectively and promote crystallinity. The bioactive components incorporated in the hydrogels exhibited anti-inflammatory and antioxidant properties. The hydrogel demonstrated a high swelling ratio, indicating good absorption of wound exudates. Furthermore, these are injectable through an 18G syringe, moldable, and exhibit self-healing properties. The contact angle measurements were consistently below 90°, confirming that the hydrogel is hydrophilic and conducive to cell attachment. Antibacterial activity was observed against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) pathogens, suggesting that hydrogel can prevent bacterial infection. Additionally, the hydrogel was found to be compatible with fibroblast cell lines and was also hemocompatible, ensuring their suitability for biomedical applications.

Keywords: *Hydrogel, Antibacterial, Biocompatible*

PP-79

Combining Mesenchymal Stem Cells, 3D Scaffold, and Small Molecule: A Tissue Engineering Approach for Cardiovascular Therapeutics

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ABSTRACT

Cardiovascular diseases (CVD) are the leading cause of death globally. In the coming decades, there will likely be a sharp rise in the incidences of CVD, particularly myocardial infarction (MI). MI causes the death of cardiomyocytes, which ultimately leads to heart failure. Medical and surgical treatments can only limit disease advancement but cannot improve the function of the infarcted myocardium. This highlights the need for a promising treatment strategy for ischemic heart diseases. Mesenchymal stem cells (MSCs) hold great potential for regenerating damaged heart tissue due to their multilineage differentiation capability and easy isolation. The use of biomaterials provides a suitable microenvironment to the cells to perform various biological functions and diminishes the poor cellular

engraftment problem associated with cell transplantation alone. Therefore, we intended to use 3D collagen scaffold along with a demethylating agent, zebularine, to enhance the cardiac regeneration potential of MSCs upon *in vivo* transplantation. MSCs were isolated from rat bone marrow, characterized, seeded in a collagen scaffold, and treated with zebularine. *In vitro* cardiac differentiation potential was assessed, and then *in vivo* transplantation was performed to evaluate the cardiac regeneration potential of treated MSCs. The results of *in vitro* treatment showed significantly enhanced cardiac differentiation of MSCs. Echocardiographic analysis revealed enhanced functional improvement while histological analysis showed regeneration of cardiac tissue beneath the scaffold, less fibrotic scar, and improved ventricular wall thickness in the zebularine-treated MSCs-seeded collagen scaffold transplanted group. This strategy will be helpful in cardiac tissue regeneration after ischemic cardiac injury. In the future, translation of this research into clinics can provide an effective therapeutic strategy for improving cardiac function and regeneration.

Keywords: *Myocardial infarction, Mesenchymal stem cells, 3D scaffold, Zebularine, Cardiac differentiation.*

PP-80

Bioinspired Angiogenic and Anti-Inflammatory Apatite-Infused Self-Healing Hydrogels for Enhanced Bone Defect Repair

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ABSTRACT

Bone grafts offer a promising solution for repairing defects caused by orthopedic trauma, infections, tumors, and osteonecrosis. This study presents the development of a novel mineral-organic bone substitute material with enhanced osteoconductivity and moldability. Inspired by the biocompatibility of biopolymers and the bioactivity of hydroxyapatite (HA), a composite self-healing hydrogel system was designed using HA co-assembled with biopolymers through borate ion crosslinking. Hydrogels were synthesized with varying HA concentrations (12%G, 33%G, 50%G, 60%G, and 67%G), and their structural and chemical properties were confirmed through Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). *In vitro* swelling and degradation assays demonstrated the stability of the three-dimensional hydrogel network under physiological conditions. Hemolysis studies confirmed the blood compatibility of the hydrogels, while cytocompatibility assays using osteoblasts

and fibroblasts indicated no cytotoxic effects. Additionally, gene expression analysis of vascular endothelial growth factor (VEGF), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) showed a minimal inflammatory response compared to controls. Furthermore, *in vivo* evaluation using a rat calvarial defect model demonstrated the potential of these hydrogels to enhance bone healing. This study helps to develop an advanced potential solution for bone repair, remodeling, dry socket treatment, post-surgical defect healing, implant site development, periodontal regeneration, sinus lift surgeries, and localized ridge defect restoration.

Keywords: *Bone regeneration, hydroxyapatite, self-healing hydrogels, polymeric crosslinking, osteoconductivity, biocompatibility*

PP-81

Root-associated Bacteria in Phytoremediator Hydrophytes: Isolation, Characterization, and Remediation Potential for Heavy Metals

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ABSTRACT

Unplanned industrialization imposed great challenges due to the release of untreated industrial waste. Chemical methods for the removal of contaminants have shown hazardous impacts on the ecosystem. Bioremediation is the natural process for the treatment of industrial waste applying plants and microorganisms. The present study is the molecular identification and chemical characterization of root-associated bacteria isolated from roots of *Pistia stratiotes and Eichhornia* growing in industrial wastewater and investigation of their bioremediation potential against different heavy metals. Plants and wastewater samples were collected from the industrial zone of Gujranwala, Punjab, Pakistan. A heavy metal analysis of wastewater samples was performed using atomic absorption spectroscopy. The physicochemical properties, including electric conductivity (EC), pH, and Total dissolved solids (TDS) of wastewater samples, were also quantified. Isolation and chemical characterization of root-associated Endophytic Bacterial DNA was isolated by the phenol-chloroform method. The molecular identification was done by PCR amplification through 16s rRNA. The bioremediation potential of identified bacterial strains was observed against different concentrations of heavy metals, including

Zinc (Zn), Lead (Pb), Nickel (Ni), Chromium (Cr), and Cadmium (Cd). The average pH, EC, and TDS were recorded as 7.24 ± 0.7424, 2089 ± 117.379 S/m, and 1336.96 ± 0.813, respectively. Heavy metals, including Fe, Zn, Cr, Cu, Pb, and Ni, were detected in the wastewater. The order of the ranges is Na>Pb>Cu>Ni. The average concentration of Na was 7680 ± 311.12 mg/ L, Cu was 0.29 ± 0.014 mg/L, Pb was 0.42 ± 0.028mg/L and Ni was 0.405 ± 0.021 mg/L. The molecular identification of root-associated bacteria isolated from *Pistia stratiotes* were *Bacillus cereus* (OQ509998) and from *Eichhornia crassipes* were *Enterobacter asburiae* (OR056289) that assist the plant in the process of phytoremediation. These bacterial strains showed resistance against heavy metals in the well diffusion method. The growing environmental challenges linked with industrial wastewater need environment-friendly remediation approaches. The bacterial species *Enterobacter asburiae and Bacillus cereus* could be used alone or in combination with other phytoremediators for rapid recycling of wastewater.

Keywords: *Bioremediation, phytoremediation, Enterobacter asburiae, Bacillus cereus, Pistia stratiotes, Eichhornia crassipes*

PP-82

Production Of Bioplastics by Using Recombinant Bacteria

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ABSTRACT

The widespread use and improper disposal of petroleum-based plastics have led to severe environmental pollution, depleting natural resources and harming ecosystems. Conventional plastics are non-renewable and non-biodegradable, posing long-term sustainability challenges. To address this issue, this study explores the use of bacteria as bio-factories for the production of biodegradable plastics, particularly polyhydroxyalkanoates (PHAs). Engineered bacterial strains, such as E. coli and purple bacteria, are optimized to convert organic waste, carbon dioxide, and other renewable resources into bioplastics through scalable fermentation techniques. These processes enhance bacterial growth and polymer production while utilizing low-cost feedstocks, reducing dependency on fossil fuels and lowering production costs. The adoption of bioplastics presents significant environmental benefits, including reduced plastic waste, decreased marine pollution, and lower greenhouse gas emissions. Their production from renewable sources like plant biomass further enhances sustainability. However, large-scale manufacturing may raise concerns regarding land-use conflicts, potentially affecting food production and biodiversity. The target market for bioplastics spans various industries, including packaging, agriculture, healthcare, and consumer goods, where biodegradable materials can replace

conventional plastics. The commercialization plan emphasizes cost-effective production methods, strategic partnerships, and sustainability certifications to attract eco-conscious consumers. Expanding production capacity, particularly in high-demand regions like Europe, and competing with traditional plastics through cost reduction are critical components of market penetration. Overall, this research highlights the potential of microbial bioplastic production as a sustainable alternative to conventional plastics, contributing to environmental conservation and a circular economy.

Keywords: *Bioplastics, Polyhydroxyalkanoates (PHAs), Environmental Biotechnology, Sustainable Packaging, Microbial Fermentation, Renewable Resources.*

PP-83

Evaluation of Azo Dyes and Plastic Degradation Potential of Fungal Strains and Their Role in Wastewater Treatment

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ABSTRACT

The biodegradation capability of *Aspergillus niger* and *Trichoderma viride* was tested on azo dyes and plastic strips. For the dye decolorization assay, two fungal strains were exploited to evaluate their degradation capability on Synozol Red, Yellow, and Navy-Blue dyes which gave the utmost decolorization such as 40%, 70%, 90% by *Aspergillus niger*, and 36%, 73%, 87% by *Trichoderma viride*, respectively for 60 days. The GC–MS analysis of the decolorized dyes suggested that various compounds such as Caprolactam, Furazan-3-carboxamide, oxime, 4-amino-N, N-dimethyl, 6H-Pyrazolo [1,2-a] [1,2,4,5] tetrazine, Hexahydro-2,3-dimethyl, Benzene, 1-propenyl, Dihydroxy maleic acid, Arsenous acid, tris(trimethylsilyl) ester were produced by the fungi which helped in the removal of dyes from the wastewater. The laccase activity of the degraded dyes was proof that both of the strains positively produced the enzyme that helped in the biodegradation of carcinogenic dyes into less harmful products. The *A. niger* extracted laccase relative activity was 262%, 265%, and 145.7% for Synozol Yellow, Synozol Red, and Navy Blue, respectively. Similarly, laccase, obtained from *T. viride*, showed relative activity of 187.5% against Synozol Yellow, 215% against Synozol Red, and 202% against Navy Blue. Furthermore, the supernatant extracted from fungi-decolorized wastewater was used to check phytotoxicity on *Vigna radiata*, which gave excellent results.

For the plastic degradation assay, the same fungal strains along with the consortium were utilized to examine the weight loss percentage of the plastic strips. The best results were obtained when a mix of

both fungal strains was used, which gave a 54.68% and 48.94% reduction in weight for PDB and CDB respectively. *T. viride* was not exceptional in this regard. The GC-MS analysis of the liquid medium extracted after incubation of fungi with plastic strips resulted in various compounds such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 1,3-Propanediamine, N-methyl, n- Hexadecanoic acid, Octadecanoic acid, etc. The FTIR analysis of the degraded plastic strips showed relative coarseness, suggesting vigorous degradation. The wavelength range was kept at 4000-450cm^{-1}.

Keywords: *Azo dyes, plastic, fungi, degradation assays, GC-MS, FTIR*

PP-84

Simulated Field Trial of CEMALGATECH-1: A Self-Sustaining Mosquito Larvicidal Transgenic Microalga Expressing Cry11Ba Protein

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ABSTRACT

Mosquito-borne diseases are one of the most common causes of epidemics globally. Mosquito larvicides play an important role in the management of the mosquito population by targeting the mosquito larvae before they mature into adults. The conventional chemical larvicides are quite effective but can lead to environmental pollution, toxicity, hazardous effects on non-target organisms, and the emergence of resistance. To combat these challenges, we have developed a genetically engineered microalga named CEMALGATECH-1 that expresses the mosquito larvicidal cry11Ba protein. CEMALGATECH-1 resulted in 100% larval mortality of 1 st and 2nd instars of Aedes aegypti, the carrier of dengue virus, after 48 hours of feeding in lab-scale bioassays. Furthermore, a simulated field trial was conducted according to WHO guidelines to assess its efficacy under more realistic conditions. CEMALGATECH-1 showed 90% larval mortality of Aedes aegypti, even at a low dose of 1x104 cells/ml under simulated field conditions. These results demonstrate the potential of CEMALGATECH-1 as an economical, eco-friendly, and self-sustaining biocontrol agent for integrated mosquito management programs.

Keywords: *CEMALGATECH-1, simulated field trial, mosquito larvicidal transgenic alga, self-sustainable, cry11Ba protein, dengue control.*

PP-85

Construction of an Enzyme-based Electrochemical Biosensor using NiCr2O4/g-C3N4- Modified Pencil Graphite Electrode to Investigate Malathion Sensitivity in Insects

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ABSTRACT

The reduced sensitivity and resistance to insecticides are related to disparate susceptibility and sensitivity of insecticides to the Acetylcholinesterase (AChE) enzyme in insects. This study aimed to monitor as well as investigate the sensitivity of an organophosphate pesticide to the AChE activity of three insects, i.e., *A. mellifera (honeybee), T. castaneum (Red flour beetle),* and *Z. nevadensis (Termite sp.),* by an electrochemical biosensor. For the construction of the biosensor, the transducer was modified with $NiCr_2O_4$/g-C_3N_4 composite that was prepared and characterized for its morphological, chemical, and electrical properties. The composite integrated pencil graphite electrodes were then covalently immobilized with insect AChE enzyme from each insect separately, and the amperometric response of the bioelectrodes was determined through cyclic voltammetry. The prepared bioelectrodes exhibited high enzyme immobilization efficiency and electrocatalytic performance. The results were most significant for *A. mellifera* AChE. The inhibition efficiency of an OP, Malathion, was tested and the linear ranges were found to be 0.1 - 1.6 µM, 1 - 40 nM, and 2 - 100 nM, the LODs were 2 nM, 0.86 nM and 2.3 nM, and LOQs were 6 nM, 2.6 nM, and 7 nM for A. mellifera, T. castaneum, and Z. nevadensis, respectively. Additionally, the biosensing platform developed using A. mellifera AChE was found highly sensitive and effective for Malathion recoveries from spiked wheat flour samples with high recovery rates. Moreover, the proposed method was adequately reproducible and selective. The results revealed that A. mellifera AChE is less sensitive to inhibition by Malathion as compared to T. castaneum and Z. nevadensis AChE. The experimental results were also validated through computational docking of Malathion with insect AChEs, and the results were in correspondence to experimental outcomes. The proposed method can be a plausible alternative to conventional analytical methods to assess the pesticide sensitivity and toxicity of various compounds against insect enzymes.

Keywords: *Enzyme based biosensor; Acetylcholinesterase; Electrochemical assay; Pesticide Detection, Toxicity*

PP-86

Bioremediation of Textile Disperse Dyes using White-Rot Fungi *Trametes gibbosa* and *Trametes versicolor*

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ABSTRACT

Because synthetic dyes are released into aquatic environments, the growing usage of these dyes in industry raises serious environmental problems. Disperse dyes, which are employed in textile dyeing, are especially resilient to deterioration. The bioremediation capability of white-rot fungi *Trametes versicolor* and *Trametes gibbosa* for the degradation of dispersion dyes is examined in this work. Through redox reactions and the generation of radicals, these fungi produce ligninolytic enzymes that aid in the breakdown of dyes. To find the best growing conditions, both fungi were cultivated on nutritional agar/broth media, PDA, and YBD. With yeast extract and beef extract as nitrogen sources, *T. versicolor* grew best at 28°C and pH 6, but *T. gibbosa* grew best at 30°C and pH 6 with beef extract. Under ideal circumstances, *T. gibbosa* outperformed *T. versicolor* in terms of biomass production. Both visual inspection and UV-visual spectrophotometry were used to evaluate the dye degradation efficiency. While *T. gibbosa* degraded to a 0.02% DR1 solution over six days (absorbance shift from 1.56 to 2.98), T. versicolor showed greater dye degradation, dropping absorbance from 0.02 to -0.11 in three days for a 0.05% DR1 solution. Both fungus degraded wastewater samples by more than 80%, with the greatest degradation taking place in six days. At greater dye concentrations, *T. gibbosa,* however, demonstrated a decrease in efficiency. According to these results, *T. versicolor* is a good option for wastewater treatment since it is more efficient at degrading dispersion dyes. Its efficiency can be increased with further enzymatic activity and ambient condition optimization, offering an environmentally benign remedy for industrial dye contamination.

Keywords: *Bioremediation, White-Rot Fungi, Disperse Dye Degradation, Wastewater Treatment*

PP-87

Whole Genome Sequencing and Metabolic Pathway Analysis of *Methylobacterium* TP4: A Novel Methanol-Utilizing Phyllosphere Bacterium

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ABSTRACT

Over the last few decades, research in atmospheric chemistry has highlighted the role of tropospheric trace gases, especially methanol, in disrupting atmospheric chemistry. Methanol is the second most abundant and reactive VOC, which generates several secondary pollutants, leading to acid rain, smog production, ozone formation, and ultimately, global warming. Various biogenic and anthropogenic sources actively produce methanol in the lower atmosphere at levels ranging from 1 to 10 parts per billion (ppb), whereas microbial uptake is the major sink. About 80% or two-thirds (100-128 Tg) of the overall methanol is contributed by plants, largely rice fields. Conversely, methanol-utilizing bacteria residing plants, known as Methylotrophs, utilize methanol as a carbon and energy source and act as natural methanol biofilters to address global atmospheric issues. Recent advancements in high-throughput sequencing technologies have revolutionized whole-genome sequencing in understanding the genetic and metabolic potential of methanol-utilizing bacteria. Our study underwent whole genome sequence analysis of methanol-utilizing bacteria TP4 isolated from rice phyllosphere to gain insights into its genomic architecture, methanol metabolism, and evolutionary relationships. The assembly and annotation of *Methylobacterium sp.* TP4 genome revealed a single circular chromosome of 6.06 Mb with a GC content of 69.9%, encoding 5,924 coding sequences. Genome annotation identified key methanol oxidation gene clusters, including those encoding methanol dehydrogenase and associated pathways essential for methylotrophy. Comparative genomic analysis revealed the metabolic versatility of *Methylobacterium sp.* TP4 and its divergence from previously characterized methanol-utilizing bacteria. This study contributes to a deeper understanding of methanol metabolism in phyllosphere-associated bacteria and provides a genomic foundation for future research on microbial adaptation, host-microbiome interactions, and methylotrophic capabilities.

***Keywords:** Whole genome sequencing, Methylobacterium, Methanol-metabolism, Key gene clusters.*

PP-88

Unveiling the Power of TiO Dopped ZnO Nanomaterial as An Effective Solution in the Leather Industry

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ABSTRACT

The surface protection of leather supplies is a major concern worldwide due to its susceptibility to microbial growth. Different methods are employed to protect leather, their results end up with environmental pollution and human safety issues. Nanoparticles with excellent antimicrobial potential

can provide sustainable protection to leather accessories. The present work represented a comprehensive investigation into the preparation and characterization of titanium dioxide-doped zinc oxide (ZnO/TiO_2 NPs) nanoparticles and their exploration as a potential antimicrobial agent in the leather industry. ZnO nanoparticles were synthesized through the Sol-gel method by the reduction of zinc acetate dihydrate *via* black cardamom seed's extract and subsequently doped with TiO_2. The optical, structural, and morphological features of nanoparticles were thoroughly scrutinized through UV–visible spectroscopy, XRD, FT-IR, and SEM-EDAX. The UV–visible spectrum showed enhanced performance between 300 and 350 nm, and various peaks of the FT-IR spectrum, i.e., 3315.53, 1566.20, 1402.25, 1340.53, 1014.56, 921.97, 690.52, and 677.01 cm^{-1}, revealed chemical bonds that prove the correct doping of TiO_2 in ZnO nanoparticles. The characteristic peaks obtained from XRD at 2Θ of 32°, 35.5°, 37.2°, 47.9°, 55.6° 63.51°, and 70° intimated to the crystal planes of (100), (002), (101), (102), (110), (103), and (112), respectively. SEM-EDAX images revealed the roughly spherical but agglomerated structure of nanoparticles with a size of 45.44 nm. Furthermore, minimum inhibitory concentration (MIC), antimicrobial potential, and anti-biofilm potential analyses of nanoparticles, against all selected microorganisms (*Aspergillus niger, Staphylococcus aureus,* and *Escherichia coli*) provided valuable insights into physical and biological properties of the nanoparticles. The clear zones of inhibition (29–30 mm) against these pathogenic strains showed the exceptional antimicrobial action of the ZnO/TiO_2 NPs. Overall, these results provide an approachable method to synthesize ZnO/TiO_2 nanoparticles, and their antimicrobial ability will prove to be beneficial for the protection of leather materials from various microbial contaminations.

PP-89

Production of Polyhydroxyalkanoates (phas) by *Bacillus subtilis* from Cellulase-treated Corncob Hydrolysate

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ABSTRACT

The increasing global demand for plastic has led to severe environmental and health concerns, including microplastic pollution and its associated diseases. Bioplastics, particularly polyhydroxyalkanoates (PHAs), offer a sustainable alternative due to their biodegradability and production from renewable resources. This study focuses on PHA production using *Bacillus subtilis* cultivated on cellulase-treated corncob hydrolysate as a carbon source. The corncob was pretreated with 1% NaOH to remove lignin, followed by enzymatic hydrolysis using *Trichoderma harzianum*

derived cellulase. The hydrolysate contained 1.0 mg/mL glucose and 0.5 mg/mL xylose and was directly utilized for PHA synthesis. *B. subtilis* was grown in a nitrogen-deficient medium for 72 hours, with samples collected at 12-hour intervals after the 23-hour mark to monitor PHA and biomass production. Different nitrogen sources were tested, yielding the following PHA concentrations (g/L): peptone (0.71), yeast extract (1.2), ammonium nitrate (0.78), ammonium chloride (3.0), and ammonium sulfate (0.21). Notably, ammonium chloride resulted in the highest PHAs accumulation, highlighting its potential as an optimal nitrogen source. The produced PHAs were characterized using spectrophotometric and FT-IR analysis. These findings demonstrate the feasibility of corncob hydrolysate as a cost-effective substrate for microbial PHA production, contributing to sustainable bioplastic development.

Keywords: *Bioplastics, Polyhydroxyalkanoates (PHAs), Corncob Hydrolysate, Bacillus subtilis, Nitrogen Sources*.

PP-90

Development of a Cost-Effective and Reliable Serodiagnostic Assay for Tuberculosis

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ABSTRACT

Tuberculosis (TB) remains a leading cause of mortality worldwide, highlighting the urgent need for rapid, accurate, and affordable diagnostic tools, especially in resource-limited settings. This study aims to develop a reliable serodiagnostic assay by designing novel fusion antigens targeting stage-specific Mycobacterium tuberculosis proteins. Using in-silico tools, multiple antigens were truncated and combined to create novel fusion molecules with enhanced structural stability and solubility. The custom-designed genes were synthesized and cloned into pET series expression vectors. Recombinant protein expression was carried out in Escherichia coli, and soluble proteins were purified using Ni-NTA affinity chromatography, followed by validation through SDS-PAGE and western blot analysis. Currently, the project is in the validation phase, where the diagnostic potential of these fusion antigens is being evaluated through enzyme-linked immunosorbent essays (ELISA) using plasma samples from TB patients and healthy controls. Several assay parameters have been optimized, including antigen coating concentration, blocking buffers, antibody dilutions, and substrate concentration to enhance assay performance. Cut-off values are being calculated using healthy controls, and initial ELISA experiments on TB-positive samples are underway to assess diagnostic sensitivity and specificity. The goal is to establish a robust and reproducible protocol capable of accurately distinguishing TB-positive

from healthy samples. If successful, this assay could offer a scalable, cost-effective diagnostic solution for TB, with future work aimed at clinical validation and potential development into a point-of-care format for broader accessibility.

Keywords: *Tuberculosis, Serodiagnosis, Fusion Antigens, ELISA, Diagnostic Assay, Mycobacterium tuberculosis.*

PP-91

Development of Indigenous Human Adenovirus 5-based Vector Vaccines against SARS-CoV-2 and Its Emerging Variants

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ABSTRACT

Severe acute respiratory syndrome *coronavirus* 2 (*SARS*-CoV-2) was first reported in late 2019 in China and is the causative agent of the coronavirus disease (COVID-19). To combat the coronavirus disease, a vaccine is urgently needed. In the current study, highly efficacious and cost-effective viral vector vaccines were developed against local SARS-CoV-2 strains and its emerging variants. For this purpose, the wild-type/consensus sequence of spike (S-perfusion) and the nucleocapsid (N-gene) of SARS-CoV-2 was amplified and cloned into shuttle vectors under CMV promoter. The generated shuttle vectors pAdTrack-CMV-S-perfusion, pShuttle-CMV-S-Prefusion HexaPro_consensus, and pShuttle-CMV-N-gene (WT/consensus) were confirmed by restriction analysis and verified by DNA sequencing. For the generation and production of S-perfusion/N-gene (WT/consensus) adenoviral vector vaccine, a linearized shuttle vector was co-transformed along with a backbone vector into the AdEasier-1 *E. coli* strain. After homologous recombination, the potential recombinants were re-introduced into recombination-deficient *E. coli* (DH5α) strain to generate the stable clones. Potential recombinant clones (pAdEasy1-CMV-S-perfusion, pAdEasy1-CMV-S-perfusion-HexaPro_consensus, pAdEasy1-CMV-N-gene and pAdEasy1-CMV-N-gene_consensus) were used for transfection and infection into Human Embryonic Kidney (HEK293T) cells. The transfection efficiency of generated vaccine candidates was monitored by tracking GFP expression and cytopathic effects under fluorescence microscopy. The produced vaccines were evaluated for immunogenicity and safety studies in a hamster model using an intramuscular prime-boost regimen. The vaccination

elicited robust immunity compared to controls (PBS and hAd5-GFP), with no organ anomalies observed, underscoring the potential of this vaccine strategy for effective immune responses against COVID-19 and preparedness for future pandemics.

Keywords: *SARS-CoV-2, HEK293T cells, Adenoviral vector vaccine, coronavirus disease.*

PP-92

Expression, Purification, and Structural Characterization of Recombinant Receptor Binding Domain of SARS-CoV-2 Spike Glycoprotein Produced in Schneider 2 Cells

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ABSTRACT

The receptor binding domain (RBD) of SARS-CoV-2 spike glycoprotein mediates viral entry into the host cell. It is essential for virus neutralization and serves as a potential target for neutralizing antibodies. Considering these facts, RBD became the primary target for the development of COVID-19 vaccines and diagnostic tools. Multiple expression systems such as bacterial, fungal, mammalian, and insect cell expression systems have been used for the expression and production of RBD. In this study, insect cell expression system using Drosophila S2 cells has been employed for the expression and production of recombinant RBD. A construct for RBD was designed and the sequence encoding RBD gene was cloned into pMT-PURO insect cell expression vector. S2 cells were stably transfected with the RBD construct for secretory expression of protein. Purification from expression culture media was performed using the hexa-histidine tag attached to the C-terminus of protein on HisTrap column. Size exclusion chromatography of purified recombinant RBD S2 using Superdex 75 column indicated the monomeric nature of the RBD S2 protein. Moreover, the secondary structure was analyzed using circular dichroism spectroscopy which showed that RBD S2 comprises of 7.10% helix, 25.30% antiparallel β-sheets, and 13.90% turns and bends. These secondary structure contents are in agreement to the previously reported recombinant RBD from Expi293F mammalian cells expression system (Ábrahám, E., Bajusz, C., Marton, A., et al,. (2024). Expression and purification of the receptor-binding domain of SARS-CoV-2 spike protein in mammalian cells for immunological assays. FEBS open bio, 14(3), 380–389). RBD S2 obtained by this method can be further used for the development of immunology assays and next-generation vaccine candidates.

Keywords: *RBD, S2 cells, SARS-CoV-2, expression system, vaccine*

PP-93

Biological Application of *Artemisia Vulgaris* Leaves Methanolic Extract against Ethanol-Induced NLRP3 Inflammasome in Mice

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ABSTRACT

This study aimed to prepare and assess the inhibitory potential of *Artemisia vulgaris* leaves methanolic extract (AVULME) against ethanol-induced NLRP3 inflammasome activation in adult albino mice. Male mice aged 8 weeks were randomly divided into three experimental groups: (1) control group treated with 0.9% normal saline, (2) Ethanol-treated group (5 g/kg), and (3) Ethanol (5 g/kg) + AVULME (100 mg/kg body weight) treated group. The study employed antioxidant enzyme assays, including peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and lipid peroxidation (LPO), along with Western blotting techniques, to evaluate the antioxidant activity and the inhibitory effects of AVULME on NLRP3, ASC, caspase-1, Nrf-2, HO-1, TLR4, and IL-1β proteins in the brains of male adult mice exposed to ethanol. Ethanol and AVULME were administered daily for two weeks. The results demonstrated that ethanol induced oxidative stress and activated NLRP3 inflammasome complex proteins in the mice brains. In contrast, AVULME significantly restored the activity of antioxidant enzymes (POD, SOD, CAT, and GSH) that were suppressed by ethanol, while reducing LPO levels, thereby mitigating ethanol-induced oxidative stress in the brain homogenates of male adult mice. Additionally, AVULME significantly inhibited the expression of phosphorylated NF-κB, leading to the disruption of the NLRP3 inflammasome complex, including NLRP3, ASC, caspase-1, and IL-1β proteins in the brains of the mice.

In conclusion, these findings suggest that AVULME can alleviate ethanol-induced oxidative stress and suppress NLRP3 inflammasome complex proteins in male adult mice. Further detailed studies are necessary to elucidate the mechanisms by which AVULME mitigates ethanol-induced neurotoxicity in both *in vitro* and *in vivo* models.

Keywords: *NLRP3 inflammasome, Artemisia vulgaris, Catalase, ASC, Caspase-1, IL-1β.*

PP-94

Study of the Modulating Effect of Antiglycation Agents on AGEs-Induced Activation of NF-κB Protein in Mouse Primary Astrocytes

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ABSTRACT

Diabetes Mellitus is a chronic illness characterized by elevated levels of blood glucose. It is caused by the aberration in the secretion and action of pancreatic hormone insulin. The constant hyperglycemic state accelerates the formation of non-enzymatic glycation adducts known as advanced glycation end products (AGEs). AGEs are heterogeneous family of molecules, formed by non-enzymatic glycation, when proteins and lipids are exposed to sugars during hyperglycemia. Their accelerated formation, and accumulation in body tissues is a biomarker for pathological post-diabetic complications such as, cardiomyopathy and neuropathy. AGEs ligate with their receptor RAGE, and stimulate reactive oxygen species (ROS) production, that in turn activates redox sensitive master switch NF-κB. The acetylation of Rel A (p65) and methylation of c-Rel NF-κB subunits play a key role in modulating NF-κB mediated cellular responses. The current study tested the efficacy of antiglycation agents that can interrupt AGEs-induced NF-κB nexus by down-regulating p65 acetylation, and up-regulating the activation of methylated c-Rel in isolated primary mouse astrocytes. The isolated primary astrocytes were characterized for their specific marker Glial fibrillary acidic protein (GFAP). The cytotoxicity of antiglycation agents was tested on primary astrocytes. All the tested compounds were found non-toxic. The efficacy of compounds against AGEs-induced ROS formation was determined via DCFH-DA assay. The compounds significantly reduced intracellular ROS formation. The potential of compounds against NF-κB translocation was assessed through immunocytochemistry. It was found that they significantly inhibited the p65 subunit of NF-κB, and up-regulated methylated c-Rel. Thus, it is concluded that the tested antiglycation agents may have the potential to treat diabetes-induced neurological complications.

Keywords: *Astrocytes, Antiglycation, Post-translational modifications, p65, c-Rel*

PP-95

Molecular Insights into hunchback mRNA Regulation in Drosophila: A Mass Spectrometry-Based Approach

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ABSTRACT

Gene regulation in Drosophila melanogaster is a complex, multi-layered process that governs development, cellular differentiation, and response to environmental cues. This regulation occurs at multiple levels, including transcriptional, post-transcriptional, translational, and epigenetic mechanisms. Post-transcriptional regulation plays a crucial role in Drosophila melanogaster development, ensuring precise spatial and temporal control of gene expression. One well characterized example is the translational repression of hunchback (hb) mRNA in the posterior region of the embryo, which is essential for proper body segmentation. This repression is mediated by the RNA-binding protein Brain Tumor (Brat), along with Pumilio (Pum) and Nanos (Nos). Understanding the interactions between these biomolecules at a molecular level is crucial for deciphering gene regulation mechanisms. Several biochemical and biophysical techniques have been developed to study nucleic acid–protein interactions. Electrophoretic mobility shift assays (EMSA) remain a widely used method for detecting protein binding to nucleic acids, offering high sensitivity but limited quantitative precision. Recent advances in high-throughput techniques, such as photocross-linking and immunoprecipitation followed by sequencing (CLIPseq) and ChIP-seq, allow genome-wide mapping of nucleic acid-binding proteins. BRAT functions as a translational repressor by directly binding to hb mRNA through its NHL domain, an evolutionarily conserved RNA-binding motif. Cross-linking mass spectrometry (XL-MS) enables the identification of direct contact points between nucleic acids and proteins, providing structural insights at the residue level. In the current investigation, mass spectrometry and biochemical assays have revealed that the positively charged top surface of the Brat NHL domain is critical for RNA binding. Understanding how Brat controls hb mRNA translation provides valuable insights into the broader principles of post-transcriptional gene regulation and its impact on development.

Keywords: *Gene regulation, BRAT-RNA Complex, Interaction analysis, Mass spectrometry*

PP-96

In Silico Analysis Reveals High Levels of Genetic Diversity, Mutation and Recombination among Mastreviruses

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ABSTRACT

In virus evolution, the role of genetic recombination is dynamic. One of the prospective benefits of such recombination is sexual reproduction in order to access the joint genetic resources of distantly unrelated and related viral species. It is evident, however, that many recombining viruses exhibit scant facts of genetic shuffling even though they have the plausible immoral genetic exchanges. In this study, full-length sequences of dicot-infecting mastreviruses and monocot-infecting mastreviruses were obtained from GenBank, and their sequence names were cross-verified according to the new International Committee on Taxonomy of Viruses (ICTV) Master Species List 2016v1.3. For phylogenetic investigation, groupings were first adjusted in MEGA6 programming utilizing MUSCLE. For deciding the percent nucleotide identity, viral sequences were adjusted by MUSCLE in the sequence demarcation tool (SDT) program. The Recombination detection program (RDP-4) was utilized to recognize likely parents and the degree of recombination in dicot and monocot contaminating mastreviruses. Genetic recombination played a vital role in the evolution of all geminiviruses, including mastreviruses. Intergenus recombination leads to the formation of new genera while intragenus recombination is responsible for the emergence of novel agriculturally important pathogens to threaten agriculture. With the adoption of novel metagenomic lines and contemporary molecular tools to mastrevirus discovery, we predict that there will be a swift surge in the recognized dicot-infecting mastreviruses diversification that should significantly boost up the tenacity by which the movement pathways and geographical origins of these viruses can be resolute.

Keywords: *Dicot infecting mastreviruses, Monocot infecting mastreviruses, Computational tools, Genetic landscape*

PP-97

Application of Various *In Silico* Tools and Software for Structural and Functional Analysis of a Glycerophosphodiesterase from *Pyrococcus abyssi*

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ABSTRACT

Organophosphate (OP) compounds are extensively employed in the agricultural sector as pesticides and insecticides. Additionally, they also find their nefarious use as chemical warfare agents. Acute toxicity of OP compounds is due to their ability to act as inhibitors of acetylcholinesterase, which can

result in seizures, tremors, nervous, respiratory, reproductive and hepatic problems in living organisms. Recently, glycerophosphodiesterases (GDPDs) emerged as useful tools for degradation of OP compounds. The current research work aims at utilization of bioinformatics tools as well as software for detailed structural and functional analysis of GDPD (Pa-GDPD) from *Pyrococcus abyssi*. Multiple sequence alignment indicated the presence of conserved catalytic histidines (His12 and His54) and metal-binding residues (Glu39, Asp41 and Glu105) in Pa-GDPD. Ratio of acidic to basic amino acids for Pa-GDPD was calculated to be 1.237 through Prot pi tool. Moreover, a ratio of hydrophilic to hydrophobic amino acids was found to be 0.398 in Pa-GDPD's protein sequence. In addition, structural properties of Pa-GDPD were also determined by using RaptorX web server. Pa-GDPD consisted of 30 % α-helix, 18 % β-sheets and 50 % coils. Moreover, 27 % residues of Pa-GDPD were exposed on the surface whereas 41 % residues were buried in the protein core. SWISS-MODEL web server was used for 3D modelling of Pa-GDPD and TIM-barrel fold was also identified in its structure that consisted of 9 β-sheets surrounded by 14 α-helices. Pa-GDPD was found be fairly stable through molecular dynamic (MD) simulations. In order to investigate the substrate affinity of the enzyme, various substrates were docked into the active site of Pa-GDPD. The enzyme was found to have the highest affinity for diazinon insecticide. Structural stability and a broad substrate spectrum Pa-GDPD make it an ideal candidate for OP bioremediation.

Keywords: *Organophosphates, toxicity, GDPD, MD simulations, molecular docking, bioremediation*

PP-98

Application of Bioinformatics Tools and Software for Comparative Analysis of Various Cytochrome P450

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ABSTRACT

Cytochrome P (CYP) 450 is a superfamily of heme-containing enzymes. CYP450s are highly important for living organisms as they are involved in detoxification of drugs, xenobiotic metabolism, thermogenesis, production of cholesterol, steroids and thromboxane A2. They are mainly present in liver and intestines but also found in small amounts in lungs, kidneys, adrenal cortex, testis, ovaries, breasts and placenta. CYP450s find their tremendous applications in bioremediation, pharmaceuticals and antibiotic synthesis. The purpose of current research work was to perform sequence comparison of CYP450s from various organisms. For sequence comparison, protein sequences of 71 CYP450s

were retrieved from various organisms including mesophiles, thermophiles and hyperthermophiles. Amino acid sequences of bacteria and archaeal CYP450 were retrieved from NCBI the Nucleotide database whereas protein sequences of animal CYP450 were derived from UCSC (The University of California, Santa Cruz) Genome Browser. Thereafter, physicochemical parameters of all CYP450s were determined by using Expasy ProtParam tool. Comparison of physicochemical properties clearly highlighted that 20 out of 71 CYP450s were found to be thermostable based on their aliphatic index (≥100). Then, 3D structures of these 20 CYP450s were built by using Robetta web server followed by models' quality assessment through ProSA and PROCHECK_NT. 3D structures of CYP450s were used to evaluate their structural properties (H-bond, π-π interactions, cation-π interactions, and Van der Waals forces). As π-π interactions are the major contributor to proteins' stability, 11 out of 20 CYP450s were selected as they had a higher number of π-π interactions. To conclude, thermostable CYP450s are present not only in thermophiles but also in mesophiles. Out of 11 thermostable CYP450s, 5 were retrieved from thermophiles, while 6 are derived from mesophiles.

Keywords: *Cytochrome P450, mesophiles, thermophiles, hyperthermophiles, sequence comparison, structural analysis*

PP-99

Computational Analysis of Potential Repellents Against Economically Important Insects

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ABSTRACT

Mosquitoes are vectors for many diseases caused by a variety of agents: bacteria, viruses, and protozoa. A quick rise in the mosquito population endangers many nations, for mosquitoes transmit many diseases. Human sweat attracts mosquitoes because of its lactic acid component. Insect repellent is defined as a substance that prevents insect bites from reaching a person's or an animal's skin, either locally or remotely. Recently, recruiting plant-based repellents again has arisen because they contain large numbers of bioactive phytochemicals that are safe and biodegradable to by-products that are not hazardous, with considerable tests for insecticidal and repellent ability. Most plants produce different chemicals to fight off their attackers, insects that consume the plant or phytophagous insects. In this study, the chief focus remains on computational assessment in the quest for possible repellents for important species of mosquitoes economically. This study focuses on the detailed intricacies of the molecular interactions between mosquitoes and two significant plants—Mari Gold (Tagetes spp.) and English Lavender (Lavandula angustifolia). The chemical constituents of these plants are suggested to

impart their aromatic properties along with mosquito repelling capacity, thus allowing this potential pathway for pest management study. This study aims to explore the interaction between mosquito proteins with the compounds found in the plant repellents, namely Mari Gold and English Lavender. Several bioinformatics tools have been employed during the research, including PDB, Dr. Duke, modeler 10.1, chimera 1.15, PubChem, Server NCBI, and MOE. Molecular docking demonstrates the interactions of mosquito proteins with phytochemicals. The results were confirmed further with density functional theory (DFT) studies. Out of the screened phytochemicals, the one with the least energy gap is antheraxanthin, which is 0.08144 Kcal/mol. Thus, this phytochemical has higher reactivity with protein and a lower binding energy gap. Therefore, antheraxanthin can be said to be the best repellent phytochemical against insects.

Keywords: *lactic acid, plant-based repellents, phytophagous insects, Mari Gold, English Lavender, antheraxanthin.*

PP-100

Targeting Drug-Resistant Pathogens: A Computational Approach Using Phytoconstituent-Based Virtual Screening

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ABSTRACT

The global rise in antibiotic-resistant pathogens, along with the emergence of drug-resistant fungal and viral infections, poses a critical challenge to global health. Virtual screening of a diverse ligand library of plant-derived Phytoconstituents is employed to identify potential therapeutic agents against key proteins in multi-drug-resistant bacterial strains, pathogenic fungi, and viruses. Essential enzymes and proteins such as DNA gyrase, β-lactamase, fungal sterol biosynthesis enzymes, and viral proteases are targeted. When 230 ligands are screened against 80 antimicrobial targets, then only 52 ligands show stable and effective interactions with the targets. These compounds were screened virtually, yielding lead ligands with high-affinity binding to key bacterial, fungal, and viral targets. Highly stable interaction with the lowest binding energy 11.63 kcal/mol is shown by Glycyrrhizin against the *E.coli* target protein, glucosamine 6-phosphate synthase. Beta pinene and 3, 5-Octadiene-2-one also show strong affinities when screened against Saccharomyces cerevisiae target protein lanosterol 14-alpha demethylase with the binding energy of -11.43kcal/mol and -11.32kcal/mol, respectively. Moreover, Hyoscine shows greater binding interactions against Chikungunya virus target macro domain with a binding energy of -11.30kcal/mol. d-alpha-pipecoline also shows greater affinities against Dengue

virus 2 target protein RNA-dependent RNA polymerase while exhibiting binding energy of -11.10 kcal/mol. These ligands demonstrated strong inhibition potential against pathogens. These phytoconstituents are further analyzed for ADMET properties and selectivity, ensuring high therapeutic potential with minimal off-target effects. The results demand future experimental validation and the development of novel therapeutic agents with broad-spectrum antimicrobial and antiviral activity.

Keywords: *Antimicrobial Efficacy, Antibiotic-resistant pathogens, Phytoconstituents, in-silico tools, Docking scores, ADMET properties*

PP-101

Balancing Trade-offs in Metagenomics: Evaluation of Metagenomic Tools for Recovering Low-Abundance and Strain-Resolved Genomes from Human Metagenomes

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ABSTRACT

In recent years, genome-resolved metagenomics have emerged as a powerful approach for directly recovering unculturable microbial genomes, particularly bacterial species and diverse strains prevalent at varying abundances in complex metagenomes. A typical genome-resolved metagenomics analysis involves two key steps: *de novo* metagenome assembly and genome binning. While multiple metagenome assemblers and binning tools are available to facilitate the process, each tool presents trade-offs, influencing the results. Additionally, due to the inherent limitations of metagenomic assemblers and genome binning tools, the effectiveness of these tools in recovering low-abundance species (<1%) and strains is also questioned. Therefore, in this study, we tested commonly used assemblers in combination with binners for their potential to recover genomes of usable quality. We particularly evaluated these combinations for recovering low-abundance species and strains present within the real and simulated human metagenome samples of varying complexities. Our results demonstrated that along with the well-reported trade-offs, metagenomics data analysis tools exhibit complementary effects, with certain assemblers performing optimally with specific binners. Consequently, we identified combinations that performed considerably well, with a minimal trade-off

effect, in recovering low-abundance species and capturing the maximum strain diversity from metagenomes. The least trade-off was presented by metaSPAdes-CONCOCT, making it the best approach for the recovery of such species from real metagenomes. Whereas metaSPAdes-MetaBAT2 can be the most suitable option for simulated metagenomes. However, MEGAHIT-MetaBAT2 captured the highest strain diversity and recovered a higher fraction of usable MAGs, making it a good choice for the recovery of strain-resolved MAGs. These findings suggest that selecting the right assembler-binner combination has a profound impact on the recovery of information from metagenomes. These results demonstrated that different combinations vary significantly in their performance, even for the same objective.

Keywords: *Gut microbiome, De novo assembly, Genome binning, Metagenome-assembled genomes, Low abundance species, Strain-resolved analysis.*

PP-102

Next-Generation Sequencing in the Future Diagnosis of Molecular Biology

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ABSTRACT

Next-generation sequencing is an innovative method of recognizing genetic mutations and changes in the sequencing of DNA or RNA. Next-generation sequencing generates large amounts of sequence data faster than the traditional Sanger sequencing approach. It is carried out in several stages, including DNA fragmentation, adapter ligation (attaching short sequences of known DNA to the fragmented DNA), which facilitates later amplification and sequencing, gene library preparation, and data analysis. Pyrosequencing utilizes a real-time sequencing process that measures the release of pyrophosphate during DNA synthesis, allowing for rapid nucleotide identification. On the other hand, Ion Torrent sequencing employs semiconductor technology to detect changes in pH associated with nucleotide incorporation during DNA sequencing. This approach is cost effective with quick turnaround times. Nanopore sequencing is an advanced method in next-generation sequencing (NGS), which does not require PCR amplification, which simplifies the workflow and reduces bias while allowing direct sequencing of DNA or RNA. DNA or RNA strands pass through a nanopore, disrupting an ionic current to identify individual nucleotides in real-time. This enhances reading lengths and improves genomic assembly accuracy, particularly for complex structures and epigenetic modification. NGS is widely used to identify inherited genetic disorders, novel cancer mutations, drug resistance, HLA typing, and

metagenomics sequencing. It is concluded that Next generation sequencing has changed the landscape in clinical settings and research studies. Using this technique, a lot of complex sequencing is reachable that was not attainable with traditional methods. Implementing NGS in clinical settings will enhance healthcare outcomes and improve patient care.

Keywords: *Next generation sequencing, genome sequencing, Pyrosequencing, Ion Torrent, Nanopore sequencing.*

PP-103

Determination of Chemical Constituents of *Melaleuca alternifolia* Oil and Their In-silico Interaction with OBP1 of *Aedes Aegypti*

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ABSTRACT

Aedes aegypti is a vector for causing dengue fever, which has killed over 0.3 million people worldwide in the past two decades. Repellent-based vector control strategies are found to be effective in reducing the spread of mosquito-borne diseases. DEET is a synthetic repellent, and its frequent application is associated with various side effects, and it has a detrimental effect on the environment. Previous studies at Kinnaird College had established the repellent activity of *Melaleuca alternifolia* oil. This study was designed to determine the major components of *M. alternifolia* oil and to investigate their repellent activity using an *in-silico* approach. GCMS analysis identified terpinen-4ol, γ-terpinene, aromadendrene, α-pinene and αterpineol as major components of *M. alternifolia* oil. ADMET analysis reveals that terpinene-4-ol and α-terpineol are safe compounds to be used as repellents. The Odorant Binding Protein 1 was used as a target protein, which is a major signaling component in the olfactory system. The binding site (X= 23.9316, Y= 18.3637, Z= 15.5183) was predicted to use P2rank software. MCULE software was used for site-specific docking, and Pyrx software was used for multiple docking. Results were visualized using LigPlot+, UCSF chimera, and BIOVIA Discovery Visualizer. Docking results of compounds were compared with results of DEET. Terpinen-4-ol showed stronger binding with AaegOBP1 (ΔG-6.6 kcal/mol) and formed a hydrogen bond with the PHE123 residue of the binding pocket. α-terpineol was also proved to be a good competitor of DEET (ΔG-6.4 kcal/mol) and exhibited a hydrogen bond with PHE123. In multiple docking analyses, terpinen-4-ol and α-terpineol showed high binding affinity with energies ΔG-7.0 kcal/mol and ΔG-7.4 kcal/mol, respectively. In-vitro repellent bioassays should be performed to validate the efficacy of terpinen-4ol and α-terpineol as repellents.

Keywords: *Aedes aegypti, Odorant binding proteins, GCMS, Molecular Docking, ADMET*

PP-104

Computational Modeling of CDC14A-Substrate Interactions Using Enzyme Substrate Trap and Substrate Phospho-Memic Mutants

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ABSTRACT

CDC14A phosphatase activity is essential for hearing and male fertility. Identification and characterization of its substrates are required to completely understand its essential cellular functions. Phosphatases have proven difficult to study and are mostly understudied. One continuing challenge is to establish phosphatase–substrate relationships, determining which phosphatase dephosphorylates a given phosphosite in a cellular protein. While human genome encodes 518 Kinases and they phosphorylate highly specific substrates, there are only 163 catalytically active phosphatases and are considered promiscuous enzymes. In a cell, a protein may get phosphorylated at one place but then move and play a role at another place. Simple co-localization studies may fail to detect such interactions; however, a substrate trap will be helpful. Conversely a substrate containing a phospho-mimic residue will not be dephosphorylated by the phosphatase and will remain trapped at the active site of the enzyme. So, both phosphatase dead mutant CDC14A enzyme and the phosphor-mimic mutant substrate can be very helpful in understanding the molecular role of CDC14A in hearing and male fertility. In this study we have performed MD simulation studies to study effect of such variants on the stability of the proteins and enzyme-substrate interactions.

PP-105

In Silico Mutagenesis of Alcoholic Dehydrogenase from *Staphylothermus marinus* for Enhancement of Stability

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ABSTRACT

Zinc-dependent alcohol dehydrogenases (ADHs) are highly important biocatalysts that play a vital role in alcohol metabolism. ADHs are ubiquitous and present in three domains of life (prokaryotes, archaea, and eukaryotes). In humans, ADHs are mainly present in hepatocytes and myocytes. Additionally, they

are well known for their medicinal significance owing to the detoxification of alcohol. The current research work aims at the utilization of various *in silico* tools for the engineering of a zinc-dependent ADH from *Staphylothermus marinus* in an attempt to improve its stability. The protein sequence of zinc-dependent ADH was retrieved by NCBI, the Nucleotide database. Robetta web server was used for constructing a 3D model of zinc-dependent ADH from *S. marinus*. The 3D model was proven to be of high quality through ProSA and PROCHECK_NT. *S. marinus* ADH consisted of three highly conserved catalytic residues: Cys37, His59, and Asp126. Thereafter, BLASTx and Hotspot Wizard 3.0 were employed to design the mutagenesis of ADH. BLASTx guided mutations were 714 whereas 10 mutations of ADH were designed through Hotspot Wizard. Out of 714 BLASTx guided mutations, 57 mutations were predicted to be stable through various bioinformatics tools. None of Hotspot Wizard guided mutations was predicted to be safe. Of 57 mutations designed through BLASTx, 17 ADH mutations were present within the active site or in its proximity. Then, 3D structures of 17 mutated ADHs were built by using Robetta followed by analyzing their structural stability through molecular dynamic (MD) simulations. Six BLASTx guided mutations (V87I, E153I, D194N, M203R, A215I, and S263E) were predicted to be highly stable through MD simulations. In short, engineered zinc-dependent ADH from *S. marinus* with improved stability may serve as an important tool for medical applications.

Keywords: *S. marinus, Zinc-dependent alcohol dehydrogenases, mutagenesis, improved stability*

PP-106

AI In the Field of Biotechnology

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ABSTRACT

Artificial Intelligence (AI) is revolutionizing biotechnology by enhancing data analysis, drug discovery, genetic research, genome editing, and disease diagnosis. AI-driven techniques, such as machine learning and deep learning, enhance the efficiency and accuracy of CRISPR technology, cancer treatment, and improvement of GM foods and livestock, thus, contributing towards healthy and better lives of human beings. AI-powered robotics is reshaping laboratory automation and precision biotechnology. Beyond medical applications, AI contributes to environmentally sustainable solutions, addressing global challenges such as bioremediation, biodegradable plastics, and biofuel. These AI-driven innovations play a crucial role in tackling issues related to waste management, air pollution, clean water, energy access, and biodiversity conservation. This thesis explores the applications,

challenges, and prospects of AI in biotechnology. Artificial Intelligence can be used in Precision Medicine: Enhancing Disease Diagnosis and Treatment in Biotechnology. Artificial Intelligence (AI) has emerged as a transformative force in precision medicine, offering innovative solutions for disease diagnosis, drug discovery, and personalized treatment plans. This research explores the role of AI-driven algorithms in biotechnology, focusing on their ability to analyze vast genomic, proteomic, and clinical datasets to improve healthcare outcomes. The study investigates machine learning (ML) models used for early disease detection, such as cancer and rare genetic disorders, and evaluates their accuracy and reliability compared to traditional diagnostic methods. The study also highlights challenges such as data privacy concerns, model interpretability, and the need for regulatory frameworks to ensure ethical AI applications in biotechnology. Through a systematic review of recent advancements, case studies, and AI-driven clinical trials, this research aims to provide insights into how AI can revolutionize precision medicine while addressing existing limitations. The findings will contribute to the ongoing efforts to integrate AI into biotechnology, ultimately enhancing patient care, reducing misdiagnoses, and improving treatment efficacy.

Keywords: *Artificial Intelligence (AI), Machine Learning (ML), Data Analysis, Genetic Research, Genome Editing, AI-Powered Robotics*

PP-107

Deciphering Rheumatoid Arthritis: Candidate Gene Discovery via Machine Learning and WGCNA

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ABSTRACT

Rheumatoid arthritis is a complex systemic autoimmune disorder that significantly impacts morbidity, mortality, and overall quality of life. This study utilizes gene expression data from the GEO database. We applied weighted gene co-expression network analysis (WGCNA) to identify key genes within significant modules, constructed a PPI network, and then leveraged machine learning algorithms to pinpoint feature genes. Various bioinformatics tools were employed, including cluster Profiler for functional enrichment analysis, gene set enrichment analysis for identifying biologically significant functions, CIBERSORT for immune infiltration analysis, and DEGGs to detect differentially expressed gene-gene interactions. Finally, FDA-approved anti-rheumatic drugs were docked against selected target regions. Our study identified IFIT3 and IFIT2 as two potential intersecting biomarkers through

MLSeq and WGCNA analysis. These biomarkers were closely associated with elevated levels of specific immune cells, particularly neutrophils, in rheumatoid arthritis patients. Gene set enrichment analysis (GSEA) revealed significant downregulation of the oxidative phosphorylation pathway. Additionally, molecular docking results highlighted Anakinra and Methotrexate as the most promising drug candidates for suppressing the expression of RA-associated proteins. Our findings suggest that these biomarkers have potential for clinical validation and could serve as a foundation for further investigation into RA-related pathways. This research may also contribute to identifying novel therapeutic targets that could influence disease onset and progression.

Keywords: *Machine Learning, WGCNA, gene expression, Rheumatoid Arthritis, Molecular docking*

PP-108

The Cosmic Imprint in Biological Systems: Bottom-up Research Focusing on Time, Gravity, and Molecular Evolution.

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ABSTRACT

Life is lived in the context of basic physical laws. Despite this underlying knowledge, the mind-bending nature of physics theories like relativity, quantum mechanics and string theory; their description of gravity and time, and its derivative influence in describing biological evolution has been mostly unexplored. This poster illustrates an interdisciplinary, bottom-up methodology to comprehend how physics has shaped molecular evolution and biological processes. Experiments on microgravity's influence on biomolecular interactions like protein folding, bacterial pathogenicity, and gene expression indicate that gravity has been an invisible force in evolutionary adaptation. Relativistic time dilation, where time is slower in stronger gravitational fields, could also have implications for molecular reaction rates, metabolism, and aging at microscopic levels. Through the unification of ideas from gravitational singularities, molecular clocks, and the evolutionary influence of physical forces, this study investigates how the structure of the universe might have subtly influenced the occurrence and operation of life itself. Not a whimsical convergence of unrelated sciences, this work attempts to shed light on the deep interconnectedness between fundamental physics and molecular biology, presenting an alternative view of the physical origins of life.

Keywords: *Molecular evolution, time dilation , microgravity, biophysics, gravitational singularities.*

PP-109

Bio- Digital Twins: Navigating the Intersection of Biotechnology, Ethics and Cybersecurity -An Overview of Digital Twins for Advanced Medical Care

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ABSTRACT

As new-generation information technologies continue to develop, integration and interaction between the physical and virtual spaces are becoming increasingly important. Digital twins are an idea that includes a "virtual" copy of reality that is always synchronized with the real operation scenario. The virtual version provides insights that guide real-world actions. This paper aims to provide an up-to-date picture of the main DT components, their features, and their drawbacks. Digital twins in biomedical research hold great potential to advance personalized healthcare. They optimize real-world performance, predict issues, and accelerate innovation through virtual replication. It is a digital model of a physical process connected to a real-world object by a “digital thread”. While digital twins have immense potential, their misuse or over-reliance can lead to significant harm. Privacy violations and data security risks loom large as these systems rely on vast amounts of sensitive information, making them attractive targets for cyberattacks. Digital twins also raise questions about consent, ownership, and control of data. Digital twins would be built using a nanoparticle-based measurement of body functions; it may also enable body hacking and the stealing of highly sensitive personal health data. This development is dangerous. It could potentially lead to the “militarization” of everything from food, air, and medicine to bodies and minds. Also, because human biology is complicated and current modeling methods aren't perfect, digital twins might not always accurately reflect how each patient responds, which could lead to the wrong diagnosis or treatment. Digital twins are much more complicated than they seen. Hence, besides many new opportunities, new kinds of cybercrime may arise. Balancing innovation with ethical and responsible use is the key to maximizing their benefits.

Keywords: *Digital twins, Advanced Medical Care, Virtual copy, Cybercrime, Personalized Health care.*

PP-110

SARS-COV-2: Molecular Virology, Epidemiology, Treatment, and Immune Responses: An Update and the Way Forward

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ABSTRACT

The unexpected outbreak of a novel coronavirus in Wuhan, China, in 2019 was the third time a highly virulent coronavirus emerged in human society. This virus not only swamped healthcare institutions but also had a significant influence on the worldwide economy. SARS-CoV-2 is a single-stranded positive-sense RNA virus that contains both structural and nonstructural proteins. It attaches to the ACE2 receptor in human cells and has its own RNA polymerase, allowing for effective replication. Although earlier coronavirus epidemics, such as SARS and MERS, have considerably improved our understanding of these viruses, effective treatments and epidemiological control measures are still restricted. This study summarizes SARS-CoV-2's epidemiology, clinical symptoms, structure, genome, and replication process. Nucleoside analogs are a viable therapy method that impedes viral replication via two essential pathways. Remdesivir, for example, is an adenine analog that integrates into viral RNA and suppresses replication. Additionally, chloroquine has demonstrated efficacy due to its combined antiviral and immune-modulating characteristics. The COVID-19 pandemic has quickly spread over the world, affecting more than 200 countries. Early epidemiological and clinical data from Wuhan have been updated to show SARS-CoV-2's different characteristics over its predecessors, SARS-CoV and MERS-CoV, notably its greater variability. Ongoing clinical trials continue to improve the understanding of the condition. At the same time, scientists are striving to create vaccines and therapeutic antibodies that precisely target the SARS-CoV-2 genome. However, these developments require comprehensive safety testing to assure their efficacy and safety.

Keywords: *SARS-COV2, Nucleoside analogs, Remedsivir, Chloroquine, Immune-modulating activity.*

PP-111

Fusion of Rv3874 with Rv2031c from *Mycobacterium tuberculosis* Results in Two Fold Increase in Serodiagnostic Potential

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ABSTRACT

Tuberculosis (TB) remains among top ten causes of death globally. TB can be effectively controlled if a highly sensitive method of reliable diagnostic ability is devised. Serodiagnosis involving polyclonal antibodies detection against antigen of Mycobacterium tuberculosis (Mtb) in serum samples can be instrumental. After the B cell epitope prediction in the native proteins, the expression of two antigenic proteins, namely of Rv3874 and Rv2031c, was optimized in *Escherichia coli BL21* cells. Evaluation based on Enzyme Linked Immunosorbent Assay (ELISA) of Rv3874 and Rv2031c TB antigens against 201 serum samples from TB positive patients showed 28.4% and 26.9% sensitivities, respectively. A fusion comprising of Rv3874 and Rv2031c gave improved sensitivity of 59.2%, which is more than two-fold the individual sensitivity of antigens. The fusion construct exhibited comparatively more sensitivity of 62.16% in the male subjects in comparison to 55.5% in the female subjects. Data obtained in the prediction of secondary structure using molecular modelling also validated our results. This fusion with greater sensitivity may prove a potential candidate for the better immunodiagnostic tool.

Keywords: *Tuberculosis, Novel epitope prediction, CHP Fusion, ELISA, Diagnostic potential*

PP-112

Investigating SARS-CoV-2 Infection in the Post-COVID-19 Pandemic Period: A Study of Viral Genomic Sequences

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ABSTRACT

SARS-CoV-2 has continued to evolve, and new variants have emerged, associated with new waves of infection. Given that the population has received COVID-19 vaccines, this indicates high rates of infectivity of new variants and their ability to escape host immunity. Therefore, it is important to recognize and identify new variants as part of ongoing genetic surveillance and public health efforts. JN.1 (a descendant of BA.2.86 sub-lineage of omicron) emerged in early 2024 as a predominant global

emerging SARS-CoV-2 variant of interest and has an additional L455S mutation in the spike protein**.** Our objective was to characterize new variants associated with the JN.1 lineage during 2024, the post-COVID-19 pandemic period. For this purpose, we conducted whole genome sequencing (WGS) on RNA isolated from 42 SARS-CoV-2 PCR-positive nasal specimens received at the AKUH Clinical laboratory in Karachi. Genomic sequences were used to compare variations with previously reported lineages and sub-lineages to identify the potential impact of the unique mutations on protein structure and possible alterations in the functionality. CZid and NextClade were used for sequence QC, consensus FASTA generation, genome assembly, alignment, and identification of lineages. We found 95% of SARS-COV-2 genomes comprise Omicron variants from 17 distinct lineages and sub-lineages. These were JN.1 (11%), JN.1.64 (3%), JN.1.61 (3%), JN.1.39 (3%), JN.1.16 (3%), JN.1.18.6 (3%), LA.1 (20%) LE.1.2 (6%), LB.1 3 (23%), LB.1. (3%), KS.1 (3%), MA.1 (3%), KP.2.3 (3%), KP.2.3.12 (3%), KP.3.1.1, LF.7.1 (3%) as well as 5% were Recombinant lineage XDK.1.2. We found the L204T novel mutation within the ORF1a gene as not yet reported globally. Further, several dynamic mutations within the ORF1a gene, including F3760L, E2982*, E1015K, G94S, F343L, N418S, A1092V, D2094N, T1437K, A968V, D877N, S3825P. In ORF1b include A434V and V2206L, and in ORF3a L95S. In the N gene, R41L, and the spike protein, V622A, S31P, T22N, Y1155H, Q493E, and N185T had not previously been reported in Pakistan yet. Notably, LF.7.1, LB.1, KP.2.3.12, KP.3.1.1, and JN.1.18.6 lineages carry additional dynamic mutations in spike protein that may enhance its receptor ACE2-binding affinity and immune evasion. As a result, the WHO declared these variants as Variants Under Monitoring (VUM) by the WHO. Genomic surveillance of SARS-CoV-2 during 2024 led to the identification of novel JN.1 lineages associated with COVID-19 infections in the Pakistani population. Their sequences were also submitted to GISAID. These findings highlight the continued circulation of SARS-CoV-2 in the population, indicating that the virus has become endemic in the current period.

Keywords: *VUM (Variant under monitoring), SARS-COV-2, JN.1, Omicron.*

PP-113

In Vitro Evaluation of the Therapeutic Efficacy of Syzygium cumini and Trachyspermum ammi against Eimeria zuernii Coccidiosis in Cattle

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ABSTRACT

Coccidiosis is caused by the Eimeria species, a parasitic protozoan that affects the enteric system of livestock. It primarily affects young calves and leads to diarrhea and even death in severe cases. It causes economic losses of about $ 400 million worldwide. The present study aimed to investigate the in vitro anticoccidial activity of aqueous and methanolic extracts of the mixture of *Syzygium cumini* (Jamun) and *Trachyspermum ammi* (Ajwain) against *E. zuernii*. The oocysts were isolated with the help of the floating technique after identification by morphometric analysis and purification by using the sugar floating technique. The sporulation inhibition (SPI) assay was performed in a 24-well microtitration plate by using various concentrations (500 µg/ml to 15.625 µg/ml) of the mixture of both plant extracts with two-fold dilution followed by the oocyst incubation. The same concentration of XP-SCOUR (a synthetic drug) as a positive control was used for comparison, and K2Cr2O7 was used as the negative control. The extracts showed a dose-dependent response in terms of the unsporulation of oocysts. The percentage of inhibition of sporulation at 500 µg/ml was 81.33%, 74%, and 87% for aqueous extract, methanolic extract of plant mixture, and XP-SCOUR (synthetic drug), respectively. The efficacy of the aqueous extract was relative to the synthetic drug, whereas the methanolic extract was significantly different ($p<0.05$) compared to the XP-SCOUR. The results showed that a mixture of *S. cumini* and *T. ammi* has potential anticoccidial efficacy against *E. zuernii*.

Keywords: *Coccidiosis, Eimeria zuernii, anticoccidial activity, phytotherapy, Syzygium cumini, Trachyspermum ammi*

PP-114

A Preliminary Study on Phenotypic and Genotypic Analysis of Gram-Negative Bacteria from Hospital Sink Drains: A Reservoir of Clinical Threats

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ABSTRACT

Globally, nosocomial infections caused by multidrug-resistant gram-negative bacteria pose a significant threat to patient and healthcare worker safety. This study investigates the prevalence and resistance patterns of multidrug-resistant gram-negative bacteria isolated from hospital drainage systems. A total of 35 samples were collected from hospital sink drains and cultured on MacConkey

agar containing varying concentrations of ampicillin. Biochemical identification, antibiotic susceptibility testing via disc diffusion, and phenotypic assays—including the Modified Hodge test, combined disc test, and double-disc synergy test—were performed. Additionally, PCR was conducted to detect blaTEM and blaOXA genes, and 16S rRNA sequencing was used for phylogenetic analysis. Among 50 bacterial isolates, the distribution was as follows: 18% Escherichia coli, 16% Pseudomonas spp., 12% Acinetobacter spp., 12% Klebsiella spp., 12% Enterobacter spp., 10% Proteus spp., 8% Shigella spp., and 6% Salmonella species. All strains were resistant to ampicillin, with 84% showing multidrug resistance. The highest resistance was observed against ampicillin (100%), while the lowest resistance was to chloramphenicol (12%). PCR analysis confirmed the presence of the blaTEM gene in 33% of the tested isolates. Extended-spectrum β-lactamase (ESBL) production was detected in 26% of strains, and carbapenemase production was observed in 12%. Phylogenetic analysis of two strains revealed 100% similarity with Enterobacter sp. These findings underscore the role of hospital sink drains as reservoirs for multidrug-resistant gram-negative pathogens, highlighting the urgent need for stringent infection control measures to mitigate the risk of nosocomial transmission.

Keywords: *Resistance, Gram-negative bacteria, Nosocomial infections, blaTEM, blaOXA, Multidrug resistant bacteria, Polymerase Chain Reaction, Extended-spectrum β-lactamase.*

PP-115

Analysis of Mutations in the Receptor Binding Domain of Spike Protein of SARS-COV2 In Borno State, Nigeria, and Its Effect on Protein Dynamics

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ABSTRACT

SARS-CoV-2, an RNA virus, exhibits rapid evolution due to mutations, prolonging its host presence; targeting Spike-ACE2 interaction is crucial for treatment. The study was aimed to investigate the impact of mutation on the receptor binding domain (RBD) of the Spike glycoprotein gene, specifically focusing on protein dynamics. Utilizing both wet laboratory and *in silico* methodologies, we conducted

a comprehensive analysis of twenty mutations in the Spike glycoprotein among SARS-CoV-2 isolates from Borno state, Nigeria. This analysis involved a comparison of the RBD sequences of Spike glycoprotein from the initial reported sequence of the Wuhan wet seafood market virus with those from SARS-CoV-2 isolated in Borno state. We identified twenty mutations in the Spike glycoprotein among the SARS-CoV-2 isolates. Five of these mutations caused alterations in the secondary structure of the polypeptide chain. Our findings further revealed that ten mutations significantly affected the dynamic stability of the RBD of Spike glycoprotein, with four causing stabilization and six causing destabilizations. Additionally, three mutations resulted in increased flexibility, while seven mutations led to decreased flexibility. This comprehensive investigation sheds light on how the virus adapts to various changes in different situations for optimal survival.

Keywords: *SAR-COV-2, Spike protein, Mutation, Protein Dynamics.*

PP-116

Antiplasmodial Activity of *Azanza Garckeana* Stem Bark Extract and Its Effect on Hematological Indices in Mice

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ABSTRACT

The medicinal plant *Azanza garckeana* has a long history of use in traditional medicine across Africa for treating various diseases, including malaria. This study aimed to determine the antiplasmodial activity of stem bark extract of *A. garckeana* and its effect on hematological indices in mice infected with *Plasmodium berghei*. Phytochemical analysis of the extract was carried out. In this study, there were six groups of 30 mice (Groups A to F), comprising of five mice per group. Group A were only given food and water with no inoculation and treatment, B were inoculated with *Plasmodium berghei* but no treatment was given, group C were infected and treated with artemether while D E F were infected and treated with 100mg/kg, 200mg/kg and 300mg/kg of *Azanza garckeana* stem bark extract respectively for 4 days. Parasitemia levels, chemo suppressive effect, and hematological parameters were assessed on four different days following the start of the treatment. Phytochemical analysis of the extract revealed the presence of flavonoids, saponins, tannins, alkaloids, and phenols, while terpenoids, glycosides, and steroids were absent. Following the treatment of the animals with the extract, especially 300 mg/kg dosage, it was observed that the parasitemia levels decreased significantly ($p < 0.05$) to 4.02 ± 0.18% compared to negative control with 33.99 ± 1.02% also achieving 89.17% chemo suppressive effect comparable to artemether's efficacy. Significant ($p < 0.05$)

increases in hemoglobin, packed cell volume, red blood cells, white blood cells, and platelets were seen in the hematological examination. In conclusion, *A. garckeana* stem bark extract exhibited potent antiplasmodial effects, possibly mediated by identified phytochemicals. The dose-dependent chemosuppression and modulation of hematological parameters underscore its potential as an alternative antimalarial therapy.

Keywords: *Antiplasmodial activity, Azanza garckeana, Hematological indices.*

PP-117

Design and Optimization of a PCR for the Rapid Detection of Foodborne Pathogen *E. coli* O157:H7

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ABSTRACT

E. coli O157:H7 is a foodborne pathogen that poses a significant global health problem and triggers serious gastrointestinal infections during outbreaks. E. coli O157:H7, which is primarily initiated in cattle, spreads through contaminated food sources like beef, milk, tainted fruits, and vegetables. The virulence of *E. coli* O157:H7 develops due to the production of several Shiga toxins like Verotoxin, VT-1 and Verotoxin, VT-2. The current study aimed to optimize a PCR assay for the rapid identification of the foodborne pathogen *E. coli* O157:H7 in milk. The study specifically targets the vt-2 gene that is known to cause more virulence because it produces severe complications, including hemorrhagic colitis and hemolytic uremic syndrome (HUS), than vt-1 for the detection of pathogenic *E. coli* O157:H7. Priorly cultured and attenuated cells (4.0 x1007 E. coli by OD600) were subjected to DNA extraction, which gave a total of approximately 1461ng/ul. This *E. coli* O157:H7 DNA was used to optimize the amplification of the vt-2 gene. After successful amplification, precisely measured bacterial cells were inoculated in the milk to optimize the DNA extraction process from Milk and to access the minimum number of cells required for DNA extraction from the milk sample. DNA extracted from the Milk sample inoculated with 4.0 x 1005 E. coli cells was successfully able to amplify the vt-2 gene, while the same quantity was undetectable on 1% Agarose Gel Electrophoresis. In conclusion, a minimum number of 4.0 x 1005 *E. coli* O157:H7 cells is sufficient to amplify the vt-2 gene specific for the recognition of *E. coli* O157:H7 in milk samples.

Keywords: *E. coli O157:H7, VT-2, foodborne pathogen, PCR, milk, rapid detection*

PP-118

Comparative Genomic Insights into Multi-Drug Resistant Uropathogenic *Escherichia coli* from Pakistan Reveal Resistance and Virulence Patterns

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is a major cause of urinary tract infections (UTIs) globally, with significant health implications, particularly in Pakistan, where multidrug-resistant strains pose a growing clinical challenge. This study aimed to characterize the genomic features, antibiotic resistance profiles, and virulence factors of publicly available UPEC strains from diverse regions of Pakistan. A comparative genomic analysis of 16 UPEC strains, including the reference strain CFT073 and our in-house isolated strain U1, revealed an open pangenome with substantial genetic diversity, suggesting a high degree of adaptability. Phylogenetic analysis clustered U1 with other isolates from Islamabad, indicating a distinct genetic lineage. A core set of antibiotic resistance genes (ARGs), including *AcrAB-TolC, blaCTX-M-15*, and *blaTEM*, was identified across the strains. However, U1 exhibited unique resistance markers for macrolides and sulfonamides. Common virulence factors such as adhesins, iron uptake systems, and fimbriae were detected, with U1 displaying a distinctive combination of P and S fimbriae and the vat cytotoxin gene. Plasmid analysis revealed a diverse repertoire of plasmids, including Col-like and IncF families associated with resistance and virulence. These findings highlight the adaptability of UPEC, driven by genetic diversity and horizontal gene transfer. The study emphasizes the urgent need for rapid diagnostic tools, improved antibiotic stewardship, and enhanced national surveillance programs to address the growing burden of UTIs caused by multidrug-resistant UPEC strains in Pakistan.

Keywords: *UPEC, UTI, MDR, Pakistan, WGS.*

PP-119

Isolation and Characterization of *Staphylococcus epidermidis* from Human Skin and Bacteriophage Infecting Them

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ABSTRACT

Staphylococcus epidermidis is frequently found on the skin and mucous membranes. It has become a significant opportunistic pathogen as it is recognized as one of the causative agents of a common skin condition known as acne vulgaris. The overuse of antibiotics for the treatment of this condition has led to antibiotic resistance so it is the need of hours to shift towards other alternatives among which bacteriophage therapy is prominent. Inflammatory and non-inflammatory acne samples were collected from females with their consent. These samples were processed for isolation of *S. epidermidis*. Different biochemical tests and colony PCR were performed for their identification. Their antibiotic resistance profile was determined. Most of the strains were resistant to commonly available antibiotic agents. Next, environmental samples were processed for bacteriophage isolation. A bacteriophage JH5 was isolated against *S. epidermidis.* The characterization of JH5 was done to check its efficacy for therapeutic purposes. A good phage titer of 1.22×10^9 was obtained after phage purification. JH5 was more active at pH 6 and less active at pH 4 and 10. There was no significant change in phage titer at -20°C and 4°C and a great decline in phage titer was observed after 1-hour treatment at 60°C. JH5 effectively controlled bacterial growth for up to 16 hours at MOI 1 and 12 hours at MOI 10. Maximum JH5 adsorption took place in 10 minutes and the latent period was 55 minutes. The burst size was calculated to be 19 P.F.U/cell. All samples for bacterial and bacteriophage isolation were processed according to biosafety rules and under aseptic conditions. This study has focused on the use of a novel approach towards treatment of common skin condition by using lytic bacteriophages and this requires further research.

Keywords: *Phage therapy; biofilms; coagulase negative staphylococci; acne vulgaris; opportunistic pathogen.*

PP-120

Phenotypic Characterization of MBL Enzymes In Urinary Tract Infection Causing Pathogens

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ABSTRACT

One prevalent bacterial infection is urinary tract infections (UTIs). People of all ages and genders are susceptible to UTIs, which are among the most common bacterial diseases worldwide. The area, population demographics, access to healthcare, and reporting habits can all affect epidemiological data on UTIs. An enzyme called metallo-βlactamase is resistant to the majority of antibiotics, especially when it comes to gram-negative bacteria. The Life Science Laboratory at the University of

Management and Technology in Lahore provided the glycerol stocks of the bacterial isolates used in this investigation. The culture was then reconstituted and screened on agar plates for the formation of MBL. Using disc diffusion techniques, antibacterial resistance against meropenem in the presence or absence of EDTA was found. Biochemical testing was used to identify and distinguish MBL manufacturers. Additionally, this study used Graph Pad Prism software for statistical analysis, namely the Chi-square test. Klebsiella spp. was the most common species in the bacterial isolates of the suspected UTI patients in the current investigation (100%). Three of the twenty-five bacterial isolates were found to generate MBL. Males had a lower percentage of isolates that produced MBL (8.33%) than females (15.3%), and the variation was not statistically significant (P=0.587).

Keywords: *UTIs, MBL, Antibacterial resistance*

PP-121

Isolation and Purification of Bacteriophages Against *Mycobacterium fortuitum*

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ABSTRACT

Mycobacterium fortuitum is a fast-growing, non-tuberculous mycobacterium (NTM) that is widely distributed in the environment, including water, soil, and dust. *M. fortuitum* is a significant cause of infections in immunocompromised individuals and poses challenges for treatment due to its resistance to multiple antibiotics. This study aimed to isolate and purify bacteriophages capable of targeting and lysing *M. fortuitum*. Different environmental samples, including sewage, agricultural soil, animal dung, cow shed soil, industrial soil and sludge, and soil near water bodies, were screened for bacteriophages using *M. fortuitum* as an indicator and host strain. Several distinct bacteriophages were successfully identified through the spot test. Lytic activity was confirmed through plaque assays, and the phage demonstrated stability under a range of environmental conditions. These bacteriophages were then purified, and lytic properties were characterized. These results highlight the potential of bacteriophages as an alternative therapeutic approach against *M. fortuitum* infections, particularly in the face of growing antimicrobial resistance. Further studies are needed to assess their efficacy in vivo and the possibility of developing phage-based treatments.

Keywords: *Non-tuberculous mycobacteria (NTM), Bacteriophage isolation, Phage therapy, M. fortuitum, Plaque assay, Phage purification*

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Isolation and Characterization of Lytic Bacteriophages Against Multidrug-Resistant Bacteria: A Strategy to Combat Antimicrobial Resistance

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ABSTRACT

The alarming rise in antimicrobial resistance has become a significant public health concern, highlighting the need for potential alternative solutions. Bacteriophages, or phages, are viruses capable of targeting and killing bacteria and have gained prominence in controlling drug-resistant bacterial infections. The overall objective of this research is the isolation of lytic bacteriophages against multi-drug-resistant bacterial strains from natural resources to assess their therapeutic potential. The purified bacteriophages are then characterized through structural, molecular, and physiochemical analyses. Six lytic bacteriophages capable of targeting MDR pathogens, including two phages (one of them is a novel phage) against *Escherichia coli*, one novel phage against *Pseudomonas aeruginosa*, two phages against *Klebsiella pneumoniae*, and one phage against *Enterococcus faecalis*, have been successfully obtained. The isolated phages were characterized using molecular and genomic approaches to ensure a comprehensive analysis. PCR amplification targeting the major capsid protein gene was carried out to confirm the identity and classification of the phages. Additionally, whole genome sequencing was conducted to obtain detailed genetic information, allowing for a deeper understanding of their structural components, evolutionary relationships, and potential functional genes. The lytic phages remained stable across various pH and temperature conditions, indicating their potential resilience in different conditions. Host range analysis revealed that the phages effectively infected various bacterial strains within their respective genera, suggesting strong lytic activity. These findings will contribute to advancing phage therapy, ensuring a sustainable therapeutic strategy for controlling antimicrobial resistance.

Keywords: *Antimicrobial Resistance, Phage Therapy, Lytic Bacteriophages, Characterization.*

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Antimicrobial Potential of *Artemisia* spp. Extracts Against Multidrug-Resistant *Shigella* and *Klebsiella* Species

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ABSTRACT

The discovery of antibiotics in the 19th century significantly improved human health and life expectancy. However, the emergence of drug-resistant pathogens has created an urgent need for alternative antimicrobial agents. *Artemisia* spp., a medicinal plant rich in bioactive compounds, has shown potential in combating pathogenic bacteria. This study evaluates the antimicrobial activity of *Artemisia* extracts against multidrug-resistant *Shigella* and *Klebsiella* species. Methanolic and ethanolic extracts were prepared from the stem, leaf, and root sections of *Artemisia* spp. The antibacterial activity was assessed using the disc diffusion method by measuring the zone of inhibition. It was found that the methanolic extract demonstrated inhibition zones of 3mm (stem), 2mm (root), and 4mm (leaf) against *Shigella* spp. While the ethanolic extract exhibited inhibition zones of 2 mm (stem), 1 mm (root), and 6 mm (leaf) against *Shigella* spp. For *Klebsiella*, the methanolic extract showed inhibition zones of 2mm (stem) and 2mm (leaf), while the ethanolic extract exhibited 1mm (stem), 2mm (root), and 3mm (leaf) for *Klebsiella* sp. Conclusively, the ethanolic leaf extract displayed the highest antibacterial activity against both *Shigella* and *Klebsiella*. These findings highlight the potential of *Artemisia* as a natural antimicrobial agent.

Keywords: Artemisia *spp., antimicrobial activity,* Shigella*,* Klebsiella*, methanolic extract, ethanolic extract, disc diffusion method.*

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Conjugation of Silver Nanoparticles with Mint Neem, and Ginger Extracts to Undergo Antimicrobial Analysis

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ABSTRACT

Nanobiotechnology offers a promising future in various fields due to its enhanced applications. In the realm of health biotechnology, incredibly small-sized nanoparticles play a vital role as biomedicines. Silver nanoparticles are well known for their antimicrobial properties, non-toxic effects, and biocompatibility. Among various metallic nanoparticles, silver nanoparticles, due to their biological

applications like inhibition of bacterial growth, disrupt cell membrane of bacteria and inactivate enzymes, play an essential role in biomedicines. This study aims to develop an eco-friendly, biosecure, and cheaper way to synthesize silver nanoparticles via the Green Synthesis approach. Biosynthesis of silver nanoparticles is an easy to use, scalable, and speedy process, free of hazardous byproducts and has minimum limitations as compared to physiochemical and traditional chemical processes. Indiscriminate use of antibiotics promotes resistant strains of bacteria, leading to greater global threats. Silver nanoparticles, however, are less prone to bacterial resistance as compared to antibiotics, offering a promising solution of this problem. Medicinal plants, along with their extracts, have the power to manage diseases by modulating biological mechanisms. In this study, medicinal plants like mint, neem, and ginger extracts are utilized for the conjugation of silver nanoparticles that overall enhance the antimicrobial activity. These extracts have antibacterial properties, which are highly effective against resistant bacteria. They have additional therapeutic properties like anti-inflammatory and antioxidant effects, making them highly effective for comprehensive treatments of various diseases. Biosynthesized silver nanoparticles conjugated with neem, mint, and ginger extract are seen to show excellent antimicrobial properties and provide sustainable alternative solutions to conventional antibiotics.

Keywords: *Nanobiotechnology, silver Nanoparticles, Green synthesis, biomedicines, sustainable synthesis.*